

nature

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How much planning is enough?

REPORTS by the OECD on national policies for scientific research and the like have never been exactly bed-time reading for most scientists. Essential raw material, certainly, for politicians, civil servants and those who study the policies that emerge, but until recently little more than titles on a bookshelf to others. All that is changing, however, as the man on the laboratory floor increasingly realises that his own future is so inextricably bound up with the policies made in London, Paris or Washington that he simply has to take an interest.

The emergence of another weighty OECD report is therefore, perhaps, the time for a few more people to become acquainted with the kind of data on science that that organisation provides. This particular report is called *Changing Priorities for Government R & D* and, be warned, you need plenty of time (and a magnifying glass) to digest it all properly; even the authors admit that "this is by no means a short report".

One of the conclusions reached in the report, and perhaps not a very startling one, is that none of the 12 OECD countries studied had anything like a foolproof system whereby the allocation of funds and their breakdown between activities could be planned in advance through an overall 'science budget' which reflected priorities laid down as part of a government's overall policy. That may sound a laudable thing to be aiming for, until one comes to realise that some 10% of the total government expenditure on research and development in France and the UK goes to the "advancement of science via general university funds" (through the University Grants Committee in the UK), that in countries like the Netherlands, Norway and Sweden the figure is between 20% and 45%, and that in Japan it is a staggering 60+%. Persuading universities successfully that such and such a list of priorities is the right one is tantamount, at least in the UK, to removing much of their autonomy, any talk of which is liable to make the new and diligent bed-time reader of OECD reports toss this latest document to one side.

This brings to mind the calls made a year or so ago by the UK Parliamentary Select Committee on Science and Technology for a Minister of Research and Development to be appointed, and the general opprobrium that was heaped on the idea. Few would support the regimentation in such a way of an area for which regimentation is so obviously inappropriate.

Leaving aside the question of whether completely planned science is necessarily good and useful science, as the OECD seems tacitly to assume, the OECD report contains some interesting revelations about the way the 12 selected countries allocated and divided up their funds for research and development. The policy-making

machinery in each country, although running true to a basic pattern, is nonetheless definitely unique, and yet most of the 12 have come to allocate much the same fraction of total government expenditure to research and development, a trend which became particularly marked in the early 1970s. In 1961, for example, the USA, the UK and Germany spent, respectively, 10.5, 8.5 and 3.7% of their total expenditure on research and development, but by 1971 these figures had become 7.5, 7.1 and 6.0%. Coincidence? Or have three quite different systems for deciding the funding of research and development come up, roughly, with the 'right' answer even in the absence of complete planning and the complete ordering of priorities.

A closer examination of the priority accorded to research and development activities such as 'civil nuclear', 'civil space' and 'defence' reveals more similarities, but also some discrepancies. For example, the authors of the OECD report have set up, for each country, a league table which places expenditures on each of 14 research and development areas in order. Comparing these league tables for the 12 countries shows that the UK, Germany, France and Italy have roughly the same ideas about how to divide up research and development funds, and that Norway, the Netherlands, Belgium and Sweden also work to their own characteristically similar lists of priorities. On a more detailed level still, however, there are of course differences even between countries with superficially similar scientific aims; France and Italy, for instance, spend 1.6 and 0.4% respectively, of total government expenditure on 'civil nuclear'. Not surprisingly, the greatest disparities are to be found in the realms of defence research, with the USA, the UK and the Netherlands, for example, spending 4.9, 2.9 and 0.3% of their total expenditure on it.

Although items such as these appear near the beginning of any government's shopping list for research, the other end of the list contains some entries which are all too often glossed over or ignored in discussing a country's research programme. One such is research and development for the benefit of developing countries. The situation is epitomised by the fact that for 5 of the 12 OECD countries surveyed, no data were available, indicating either that a negligible amount was spent or that the amounts were hard to pick out from the general research and development background (and therefore hard to plan). France and the USA spend most, some \$20 million each in 1972, which represents 1.5 and 0.2% respectively, of the total government funding for research and development—not really too impressive when the target set for the UN Second Development Decade is 5%. □



A new way to slay old pests

Tests are under way at the Microbial Research Establishment, Porton Down, to determine the safety of two viruses which may be used in the control of insect pests. News of the work, which is being carried out under contract for the Ministry of Overseas Development, was given by Dr T. W. Tinsley to members of the British Association, gathered for their annual meeting at Guildford last week. Dr Tinsley, who is Director of the Natural Environment Research Council's Unit of Invertebrate Virology at Oxford, described the potential of viral control to Peter Collins.

AWARENESS of the potential of the virus control of an insect pest dates from the 1930s. At that time, the European spruce sawfly, *Gilpinia hercyniae*, which was causing catastrophic damage to spruce forests in Canada, began to decline in numbers and within a few years was no longer an economically important pest. The reason was found to be infection by a virus, which in Europe was one of the factors controlling the natural sawfly population. But development of the possibilities opened up by this fortunate incident was delayed by the Second World War and then inhibited by the discovery of the vast range of new chemical pesticides which set the pattern for pest control in the immediate post-war era. This pattern was to last at least as long as the commercial profitability of the chemical pesticides, especially when it was found that the research required to develop viruses for this purpose was possibly as costly, and certainly as time-consuming, as the development of new chemical agents.

There are several reasons why the situation has changed in recent years. Initially, increasing resistance on the part of pests of agricultural as well as medical importance drew attention to the advantage of all types of biological control. Further pressure in the same direction resulted from increased concern at the environmental effects of many "classical" pesticides. More recently, two even more potent factors have given impetus to all research in this field: first, the growing serious-

ness of the world food situation, and hence the urgent need for more efficient control of all manner of pests and diseases, and, second, the enormous and sudden rise in the cost of all chemical products.

The part that may be played by viral agents in this new era of pest control, and the reasons why the advance into this field must be gradual and cautious were clearly stated by Dr Tinsley in his contribution to the symposium on the work of the research councils at Guildford last week. As Director of NERC's Unit of Invertebrate Virology (UIV) at Oxford, Dr Tinsley indicated what has been achieved so far and gave some idea of what may be expected in the next few years, at least within the unit's terms of reference.

For reasons of safety, and in particular because of the risk of cross-infection with higher animals, the WHO and the FAO have recommended that among the seven groups of viruses known to occur in insects, only the baculoviruses should be considered as pesticidal agents. So far as is known, these have neither chemical, physical nor biological properties in common with any virus found in vertebrates or plants, thus limiting the risk of cross infection to other insects or, at worst, invertebrates. Within this group, the unit's work is at present concentrated on the nuclear polyhedrosis viruses (NPVs) which attack four members of the Lepidopteran genus *Spodoptera*. The larvae of these Noctuid moths are now among the world's most serious pests, attacking a wide range of important crop plants from grass to fruit trees in Asia, Africa and across the Atlantic. In particular, interest has been concentrated on *S. exempta*, the East African army worm, possibly the world's most serious grass-land pest. Able to produce up to 11 generations in a single year, it is found from southern Tanzania to Ethiopia, and its outbreaks have been known to denude up to 300 square miles of pasture in a few weeks. Not surprisingly, this work is being actively supported by the Ministry of Overseas Development (MOD), and the unit works in close collaboration with MOD's Centre for Overseas Pest Research.

One of the major problems in the use of viral pesticides hitherto has been the lack of precise information about the nature and characteristics of the viruses being used, and it is towards filling this gap that the unit's programme is directed. Its prime objectives were defined by Dr Tinsley as "the investigation of the chemical, physical and biological properties of insect viruses, and then determination of the effects on the insect hosts and the manner in which the viruses spread under natural conditions". The first of

these objectives involves fundamental research in a field where little such work has been done, and in fact the unit is here leading the world.

To be of value in developing any virus as a biological control agent, four distinct steps have to be taken, and these too have been precisely defined. They involve:

- The isolation, purification and detailed characterisation of each virus in such a way as to enable its unequivocal identification. (It is surprising that this has not even been done previously for the viruses which are already in use as pest control agents in various parts of the world.)
- Testing the purified virus under laboratory conditions to assess its efficacy and establish its host range.
- Testing the toxicological properties of the virus "together with any associated formulative materials". This must be accompanied by investigation of the possibility of infection of, and replication in, non-target invertebrates and vertebrates.
- Field trials, on an increasing scale, on the crop and in the areas in which it is intended to use the virus as a pesticide. To do this final step satisfactorily, some system of monitoring needs to be evolved, and such monitoring will have to be continued for several years. It is thus apparent that the whole process is a lengthy one, and any idea that viral pesticides can provide an immediate panacea, even within the range of pests against which their use is intended, has to be forgotten.

Only the first two steps are the responsibility of the UIV at Oxford, and already some of the results are apparent. Thus, examination of the viruses of the four species of *Spodoptera* has indicated that these viruses, although each distinct and recognisable, fall into two groups or serotypes, each with several closely related strains. This has gone some way towards answering the question whether or not, as has hitherto been taken for granted, insect viruses are specific—a matter of the utmost importance if any such virus is to be widely distributed in a natural environment. Moreover, again to quote Dr Tinsley, "this is the first time that such a detailed investigation has been undertaken and also the first time it has been possible to make unequivocal identifications of any of the baculoviruses". Moreover, until this had been done, "it was not possible to be certain that the host insects had died from the test virus, from a second unrelated virus which occurred as a latent infection, or from a cross contamination"—points that have needed to be ascertained before the second step could be undertaken with any confidence.

When sufficient purified and identified material of any of the viruses is available, it passes to the Microbiological Research Establishment (MRE) at Porton, working under contract from the MOD. One of the problems that is arising at this third stage is that although the MRE has the necessary facilities for undertaking any tests that may be required to check the toxicity of the viruses for vertebrates, their staff have no experience in handling, or working with, insects—and any results from Porton will need to be checked against the insect host. Arrangements are therefore being made, with the cooperation also of the Medical Research Council, for the closest possible collaboration between MRE and UIV at this stage. Only when a virus has been cleared of any potential ecological hazard at the laboratory level can the fourth stage of field testing, in the areas where its use is envisaged, be started. The first candidate to reach this stage is likely to be the virus affecting *Spodoptera exempta*, if only because of the MOD's very special interest in protecting the grasslands of the Commonwealth countries (and others) of East Africa. Meanwhile, however, research into the ecology of an active, natural outbreak of an insect virus is also being carried out by staff from the UIV. The insect concerned is, again, the spruce sawfly, *Gilpinia hercyniae* an outbreak of which was identified some years ago in the Forestry Commission's Hafren Forest in Montgomeryshire, UK. Dr Tinsley was already applying for permission to experiment with the relevant virus at Hafren, when, in 1970, a natural infection was discovered in the forest, which thus became an ideal field laboratory for research into the ecology and epidemiology of a natural insect virus outbreak.

Although in the UK this fundamental approach to the development of viral pesticides has been adopted and is being rigorously pursued, the same is not true elsewhere. Viral pesticides have for some time now been on the market in the USA and they are known to have been used extensively for some years in the USSR, particularly against pests of forest trees and certain industrial crops. What is happening in Russia is not at all clear, nor is it known what precautions are taken or what problems, if any, have arisen. But certain of the difficulties that the pesticide industry may face, when it comes to commercial production of a virus that has successfully passed through all four preliminary stages, have recently become evident from American experience. The viruses concerned are again those affecting certain lepidopterous larvae, namely the genus *Heliothis*, including

H. zea (on cotton, and probably the UIV's next target insect), *H. virescens* and *H. armigera*, pests of tobacco and maize respectively; the area on which *Heliothis* NPV has been used is said to be as much as five million acres. Successes in field trials on a vast scale led, in 1971, to a "temporary exemption from tolerance requirements" on the part of the ES Environment Protection Agency, and when this was later confirmed, the way was clear to go ahead with commercial production. Two companies, International Mineral Corporation and Nutrilite, put viral pesticides on the market—Viron and VHZ respectively—confident that they had at last achieved a breakthrough. Prolonged testing and field use had indicated that the degree of control was at least as good as that with chemical insecticides, whereas after 12 years of experimentation there was said to be no change in the specificity of the virus and no sign of any resistance on the part of the host. And yet, within recent months, the product actually put on sale has seemed to be less effective than those years of testing had led the manufacturers to believe. The extent to which this is so, why it should be, and how it can have happened, is not clear.

While these and other developments are going ahead in the UK and the USA, no one, in the UK at least, believes that rapid and sensationally successful control of the target pests is just around the corner. Problems will certainly arise at higher altitudes for example, unless some way can be found of getting over the undeniable fact that NPVs are inactivated by ultraviolet light (although one recent report indicated the reverse effect for the virus of one Lepidopteran, *Autographa californica* at least when grown *in vitro*). Then there seem to be problems relating to the effect on the virus of various leaf surfaces. A great deal of work will have to be done on the evolution of suitable formulations for large scale manufacture and application of the commercial product—especially in view of recent American experience. Finally, there is some uneasiness about the adequacy of the protocols for safety testing, so far developed by the WHO on the basis of those used at present in the United States—and themselves developed for non-replicating, easily identifiable chemical pesticides.

But once these new pesticidal agents become available, unequivocally identified and rigorously tested, in standardised formulations that can be used in complete safety, a new and most powerful weapon will be available against many of the pests that threaten the world's essential food supplies and industrial crops. □

KENNETH MELLANBY



Mathematics out of molehills

THERE is generally an optimum period for any worker to continue in a particular field of research. It takes a little time to become familiar with a new topic, productivity may then build up, but eventually he becomes stale and the "law of diminishing returns" seems to ensure that progress becomes slower and slower. This is the time to change direction, though after a fallow period the field may once again become productive.

These musings were prompted by a reader who writes: "Last week I was staying on a farm and looked at mole tracks as the moles went about ruining a newly seeded lawn. Why do the tracks so often run at right angles; they seem to turn through 90° at various points along their track. Perhaps they are particularly geometric beasts". Now I have been interested in moles for years and have even produced a monograph on the subject, but familiarity has evidently prevented me from recognising the interest of the tunnel pattern. My immediate reaction on receiving the letter was to go out and see what wild moles were actually up to. I looked with new interest at the working in my own garden, four acres of near-wilderness where wildlife is encouraged. I also returned to my main study area, Monks Wood National Nature Reserve. I may perhaps remind readers that moles, like most of our native fauna, are primarily woodland creatures, though they have been able to adapt to man-made habitats like farmland and lawns. I found everywhere, and clearly recorded in my own data made in previous years in farmland, that they do indeed have a predilection for right angled bends in their tunnels, particularly in new excavations in cultivated soil. In the wood, where they live in permanent tunnels which house generation after generation of solitary moles, this pattern is not so noticeable, possibly because of modifications over the years where repairs have been executed.

Another observation revealed something which may also be a function of

I write to you from a Soviet camp for political prisoners, located in the Lesnoy Settlement in Mordovia (Camp ZhKh-385/19). The problem to which I would like to draw your attention is of vital interest not only to me personally, but also to a large number of Soviet political prisoners—scientists who are in camps for expressing their political views openly.

In this letter I will not concern myself with whether and up to what extent the persecution of citizens for exercising their right to free speech, guaranteed to them by law, is lawful and justified, though this fact in itself should also come to the attention of world scientific opinion. However, this question is too vast for a thorough examination in a letter. Furthermore, it is up to each individual scientist to decide whether he wishes to express himself on this subject or not.

I would only like to draw your attention to the conditions in which these political prisoners are held and how this affects their professional skills. Among us there are physicists, mathematicians, biologists, civil engineers, philologists, philosophers and many others. Personally, I am an astronomer, specialised in the field of meteoric astronomy and astro-biology. Each one of us, regardless of his political views, remains, above all, a scientist and aims to preserve his professional skills under any circumstances.

However, this is impossible under the conditions which prevail in the camp. A specialist who is sent to a camp is deprived of the opportunity to follow developments in his field effectively. In accordance with Soviet law, to receive literature, periodically or in any other way, published abroad is categorically forbidden. This ban extends to

literature of non-political content, including specialised literature and literature published in countries on friendly terms with the USSR. Subject to so many restrictions and in practice totally isolated from any source of information, a scientist soon feels he lags behind contemporary science and that he is losing his professional skills. In accordance with existing forms of evaluation, total disqualification is reached in approximately three years.

Letter from the Soviet Union

We are serving sentences of five, seven years and longer. We face total creative impotence. We are not just deprived of freedom for a given period of time. We are deprived of our chosen work and our profession forever.

These restrictions are imposed not only on publications from abroad. Literature published in the USSR can be received only through specialised shops, which deal by post. According to the rules operating the book market in the USSR, in practical terms this means that one can acquire literature which has been published only recently and even then only if it is in small demand and cannot be sold over the counter. Any monograph or reference book indispensable to work which has been published a few years back is impossible to acquire. To receive literature from private individuals (even from relatives) is strictly forbidden. Fear of the written word reaches such proportions that even cuttings from the Soviet press are not allowed in the mail. To receive scientific infor-

mation in private letters from colleagues—such a fashionable method during the Renaissance and widely used at present—is also impossible for us. Probably as a result of inadequate education of those who guard us during our imprisonment, letters of such ‘strange’ content arouse special suspicion. Their censoring takes many long months and quite often they are not even delivered. Letters from abroad excite particular apprehension.

The disqualification of scientific workers is speeded along by purely physiological reasons. We are all forced to do heavy physical work, and many of us who are no longer so young are badly equipped for it. This leaves us no time or energy for intellectual pursuits. The food is highly insufficient, low in calories and in quality (lacking in protein, carbohydrates, phosphorus, vitamins). A condition of semi-starvation is normal for us. I will not dwell on this problem further as it has already been examined repeatedly. I will only add that all this contributes in bringing about a sharp decrease of intellectual potential, and a weakening of memory, and so on.

For the sake of humanity and professional solidarity, I beg you, and through you scientists throughout the world, to intercede on our behalf in this difficult situation. I appeal to you to focus your attention on the plight of your colleagues. There is no need for political action. We only ask you to secure for Soviet scientists the right of unhindered use of scientific literature, and the right to maintain scientific contacts. Please send your colleagues in prison camps books and journals and other scientific material, and in this way ease the conditions in which they are held. —K. A. Lyubarskii

this same geometrical behaviour. Monks Wood consists of blocks of woodland, divided by grassy rides of short turf. The moles spend most of their time in an anastomosing network of permanent burrows under the trees, but this system is joined at frequent intervals by mole runs which cross under the rides. These almost always run straight across the ride; they never run along it. And the burrow comes out of one woodland block and enters the other, at almost exactly ninety degrees.

How and why this happens is difficult to understand. Why does the mole never make a right-angle under the woodland ride, which may be several yards across? Under a lawn, which the ride resembles, a zig-zag would normally occur in this distance. A mole busily burrowing underground in the wood

must, somehow, recognise that it has reached the edge of the ride, and it must receive some stimulus which causes it to burrow in a particular direction towards some goal on the other side.

My correspondent raised another, more familiar, problem. He asked: “How can one decently remove moles when they are wrecking your lawn without actually putting them to death?”. This is difficult, and I usually try to persuade people to learn to live with their moles. Especially in a clay soil a mole (there is usually only one at a time in the average garden) makes a series of hills from the spoil as it digs its tunnel system, but when this is complete there is little further evidence of its presence. The mole is territorial, and will keep other individuals away.

There are various repellants on the

market. If they work, they usually divert the animals to a neighbour's garden. Generally they are unsuccessful, or they only close up a section of the burrow system. The Caper Spurge, as attractive plant in its own right, is reputed to keep the garden free, but it seldom does.

If people must be rid of moles, they have to kill them. The commercial scissor trap will catch one troublesome beast, and worms baited with strychnine will wipe out a large population. But the garden, particularly if adjacent to a wood, will always be liable to re-invasion. The same will happen if the neighbours find an effective repellent! It is best to rely on the territorial instincts of your own moles, hoping that they will do little more damage and will keep out all other members of their own species. □

international news

Tbilisi: a conference with problems

from Vera Rich

THE problem of the participation of Soviet scientists in international conferences is a long-standing one, and organising secretaries have for many years had to deal with the problem of delegates who are refused visas and substitutes who have little or no knowledge of the subject concerned but who are "approved" by the Soviet government. The case of the International Conference on Artificial Intelligence at Tbilisi this week raised the problem in a particularly acute form. Since the venue of this conference was in the USSR, the difficulties over visas and "exit dossiers" could not arise for would-be participants within the Soviet Union. Nevertheless, several Jewish refusenik scientists have been prevented from attending.

Professor Aleksandr Lerner was informed on June 5 by the KGB that it regarded the invitation from the Programme Secretary of the Conference, Professor Patrick Winston, as an "American provocation". Since then Professor Lerner has been called in by the KGB and questioned for several hours in connection with his invitation to the conference.

Another would-be participant, Ovsei Gel'man, received his long-awaited visa for Israel just in time for him to be safely out of the Soviet Union before the conference began. On reaching Vienna, he disclosed the remarkable piece of information that the physicist brothers Isai and Grigori Gol'dshstein, who, being residents of Tbilisi would not have to obtain any travel or residence clearance to attend the conference, still had no idea at the end of August that they had been invited. It should be noted that these tactics of exclusion originate from the KGB only; the "establishment" Soviet scientists themselves have made no objection to the participation of the refuseniks.

The Tbilisi conference has been used by more than 70 academics from British, US and Canadian universities as an occasion to protest against the treatment of Soviet scientists Valentin

A NEW Soviet base is to be established in Antarctica this year to prospect for mineral resources on the continent. Manned by a research team of 50 geologists, geophysicists and cartographers the station, called Druzhnaya Amity, will be based on the Filchner Ice Shelf at the southern end of the Weddel Sea, a region that until now has received scant Soviet attention.

According to an announcement from the official Soviet news agency, Tass, exploration will extend from the Antarctic Peninsula to Queen Maud Land, crossing an area which, until territorial claims were suspended by the Antarctic Treaty in 1961, was a part of the British Antarctic Territory that had also been claimed by both Argentina and Chile.

There is little room for doubt that the prime objective of the new Soviet team will be to prospect for mineral resources, with oil, gas and copper as the main targets. The Tass announcement stated that "the south [of the region to be surveyed] is reminiscent of the ore bearing zones of Siberia, while the west is a continuation of the American mountains [Andes] famous for their deposits of non-ferrous minerals.

"The most extensive region of continental shelf in Antarctica, a potential natural storehouse of oil and gas," the statement continued, "extends across the Weddel Sea and the Filchner Ice Shelf". Significantly, Soviet interest in Antarctica has in the past been concentrated largely on the opposite, eastern side of the continent.

Although official sources in Britain deny that there is any significance in the timing of the announcement, which followed closely the eighth Consultative Meeting between the signatories to the Antarctic Treaty in Oslo, it may force a decision on the

outstanding problem of the commercial exploitation of Antarctica. The Oslo meeting, which ended on June 20, was the first at which the signatories confronted that issue face on, and they were unable to reach any final decision. It was, however, agreed that the matter would become the subject of exhaustive international investigation involving all 13 signatories, and to that end a meeting is to be held in Paris in 1976 in preparation for the 1977 Consultative Meeting in London.

The Soviet decision to press on with preparations for eventual commercial exploitation may, however, precipitate a more positive response because of the new station's location. Sovereignty over the Filchner Ice Shelf was formerly the subject of an unsettled dispute between Argentina, Britain and Chile, and though territorial claims are suspended under the treaty, awkward legal questions would emerge should the Soviet Union uncover commercially exploitable resources in the area.

The decision to establish the base does not apparently run contrary to the Oslo recommendations and it is unlikely that the Soviet government will act to contravene the terms of the existing treaty. Though a solution to the problem remains some way off, there are indications that the signatories may eventually reach a mutually acceptable solution within the treaty, particularly as they are reluctant to throw the matter open to broad international debate. It is, therefore, likely that the Soviet Union, while attempting to forestall further prevarication on an issue of growing concern, will uphold the treaty; certainly, there is no reason why under its terms they should not establish a base in what was once British Antarctic Territory.

—Allan Piper

Turchin and Leonid Plyushch. The Western group, which includes many eminent names in the field of artificial intelligence, have petitioned the Soviet Academy of Sciences, Premier Brezhnev, Procurator General R. Rudenko and a good many more prominent officials. Noting that Turchin and Plyushch have been dismissed from their jobs and harassed "for defending the freedom of their colleagues", the

signatories proclaim Turchin's dismissal as "inexcusable" and Plyushch's hospital detention and drug treatment as "horrifying".

"We want to make it clear", they add, "that scientists around the world, and in particular members of the Artificial Intelligence community, have taken a keen interest in the fate of these men, and strongly protest against the treatment they have received."

Turchin is a cybernetician and physicist responsible for designing and implementing the language REFAL, which is used for designing other languages and for proving the correctness of programs. Jointly with Andrei Sakharov he co-authored a memo on human rights and social problems in 1970, and in September 1973 he circulated an open letter protesting against the official harassment of Sakharov. This led to his expulsion from the Moscow Institute for the Automation and Construction Industry in July 1974.

Leonid Plyushch, whose case has already been reported in *Nature*, was dismissed from his job at the Cybernetics Institute of the Ukraine Academy of Science after publishing a letter of protest against the trial of Yuri Galanskov and Alexander Ginsburg. He was arrested in January 1972 on charges of anti-Soviet activity and, after examination at the Serbsky Institute of Forensic Psychiatry, was diagnosed as suffering from "creeping schizophrenia with messianic and reformist ideas." He was not allowed to attend his own trial in January 1973, when it was decided that he should be detained in a psychiatric hospital. He remains there.

● Following the prestigious success of the Soyuz-Apollo project (at least as a publicity venture in support of détente and cooperation), the Soviet Union is pressing forward with a number of other international projects. At the beginning of August, the coopera-

tion of the space mission was repeated on a smaller scale with a joint stratospheric probe, consisting of two canisters of instruments—one Soviet and one American—mounted on a single triangular frame and carried aloft by an automatic balloon system. The apparatus, launched from a site near Ryl'sk in the Kursk region, reached an altitude of 30,000 m before descending by parachute, and the whole flight, from launch to sighting by the recovery helicopter, lasted 142 minutes. The purpose of the experiment was investigation of the stratosphere and the effect of stratospheric processes on climate and weather.

This, however, hardly seems a field in which a joint flight is needed—surely as much would have been achieved if the Soviet team had made the results of their research available to the Americans? The only purpose for such a joint flight would be the joint calibration of two sets of instruments preparatory to a full programme.

● Some cooperative ventures, of course, clearly demand the participation of all partners. Thus the programme of seismic sounding of the Pamir-Himalaya fold, using charges of TNT exploded under the Kara-Kul' (USSR) and Attok (Pakistan) salt lakes, and at the foot of Mt Nanga-Parbat (India) is a clear case for participation by scientists of the countries concerned. During the 1974 tests all three countries were, indeed, represented. In the latest set of soundings (last month), however, a further parti-

cipant was added—Italy. It is difficult to find an explanation for this addition, other than at the diplomatic level. Italy is not particularly noted for work in seismic sounding and, to date, the principal Italian interest in the Himalayas has been that of mountaineering. The participation of individual scientists in the research projects of other countries, particularly when their own does not support a similar programme, has long been urged as a productive means of international cooperation in science. The Soviet system of centralised control of science means, in effect, that any such participation must be approved by the relevant government department, and therefore is apt to be presented to the world not as the small number of foreign scientists joining a given research team but as a major diplomatic issue.

● The influence of the Lysenko period in Soviet biology still, from time to time, appears in scientific and popular articles. One such echo of the past appeared in a recent *Pravda* story (August 25, 1975) on the "Party news" page, describing the heavily laden orchards attached to the Bryansk Machine-construction Plant. These orchards, it is claimed, "were originally planted with the participation of I. V. Michurin. The workers of the Bryansk plant turned to him for advice on how best to grow fruit trees." Michurin was, in fact, noted for his work on the acclimatisation of fruit trees, although many of his methods savoured of folk lore (watering the trees with sweetened water to teach them to bear sweet fruit, for example), but after his death in 1935, when Lysenko claimed him as a forerunner of his own peculiar views on plant selection, the name Michurin became synonymous with the anti-heredity school of biology. At a time when a new drive is being made in Soviet research towards investment of time, funds and talents in molecular genetics, the reappearance of the name Michurin, cited without explanation, as a kind of cult-figure to whom the success of the trees is attributed, strikes a somewhat odd note.

● The latest monitoring device recommended by Soviet experts on radioactive pollution in seawater is the sprat. According to the findings of an extensive study made in the Atlantic and off-shore seas of Europe, the sprat is highly sensitive to products of radioactive decay possessing a long half-life, notably caesium-137 and strontium-90. According to the research team, the sprat absorbs even microscopic admixtures of these isotopes quantitatively, with an accuracy comparable with that of the latest electronic equipment. □

BECAUSE of a failure on the part of its international Board of Management to agree on the future funding of the OECD Dragon High Temperature Reactor Project, formal steps will have to be taken early this month to run the project down. Formal, because nobody really thinks that an agreement will fail to be struck before notices to many of the 320 people who work on the project (most of them seconded from other organisations) expire in December.

Much of the blame for the delay, it seems, falls squarely on the British Department of Energy which is dragging its heels on the grounds that work on high temperature reactors (HTRs) has, as far as the UK is concerned, been overshadowed by the decision to go for the steam generating heavy water reactor as the basis of the next generation of nuclear power stations. The department is reluctant to continue contributing some £1.4 million a year to Dragon through the UKAEA, even though there is a considerable hidden bonus in that the UKAEA itself claws back £2.1 million a year from the Dragon Project (total budget £3.8

million a year) for services and so on provided at Winfrith, Dorset, where the Dragon reactor is situated. It is not clear that the overall costs of Winfrith could be cut by nearly £2.1 million a year if Dragon were to disappear.

Dragon has been running for 11 years during which time it has provided valuable operating experience of high temperature reactors both for the seven members of the project (six European countries together with Euratom) and for countries like the United States, where General Atomic, for example, is actively interested in commercial HTRs. Also, recently, there has been a surge of interest in the use of HTRs as sources of process heat for the chemical and steel-making industries. European steel-makers have even formed themselves into the European Nuclear Steel-making Club (ENSEC).

Clearly the UK's partners in the Dragon Project see it as a useful thing to persevere with for a further five-year period, though admittedly the UK itself might reasonably hope for some token reduction in the fairly large share of the bill which it picks up at present.

—Roger Woodham

W. C. FIELDS once remarked that when he visited Philadelphia, it was closed. The same remark could equally well be applied to Washington during the entire month of August this year. Congress has been out of action, President Ford and some of his top advisers have been taking things easy in Colorado, and many federal bureaucrats have been off on their summer holidays, leaving the tourists to face a heat wave and record-breaking pollution levels. The big news story has been whether or not oil prices will be decontrolled, and many esteemed journalists have been driven to writing about how dull it all is. Quite a change from last year.

But at least one body has been keeping busy. The International Cultural Foundation, an organisation linked to the Rev. Sun Myung Moon, a millionaire evangelist from South Korea, has been putting together a meeting called the "Fourth International Conference on the Unity of the Sciences", and a glittering array of scientific and academic talent has been signed up to take part in the event. The conference, which is set to take place at the Waldorf-Astoria Hotel in New York on November 27-30, is already generating some controversy.

A similar gathering was staged in London last year. It was also stiff with Nobel Laureates and it, too, generated considerable controversy because of Moon's association with the event.

Moon is the founder of the Unification Church, a religious cult founded on an anti-communist philosophy, and supported by street-corner fund raising and by the profits of several Korean industries. Moon, who is said to be close to the South Korean dictatorship, generated some attention last year when he led his followers in prayer on the Capitol steps for the political survival of President Nixon. And his church has recently been in the headlines thanks to a court suit brought by the parents of a teenage girl who are attempting to reclaim their daughter from its clutches. They claim that the church has so much psychological influence over her that she cannot exercise her free will.

The Fourth International Conference on the Unity of the Sciences has, however, attracted a list of sponsors and advisers which reads like a page out of the *Who's Who* of science. A letter sent with invitations to the meeting lists as chairmen or advisers Eugene Wigner, Alvin Weinberg, Kenneth Mellanby, Edward David, Werner Heisenberg, C. P. Snow, Jean Piaget, Julius Axelrod, Herman Kahn, Jonas Salk, and many others. But, according to an article in *Science and Government Report*, several of the participants listed in the letter are considering drop-

ping out. They were either unaware of Moon's connection with the International Cultural Foundation when they accepted invitations, or they are simply having second thoughts about being associated with an event sponsored by Moon's organisation. Similar problems arose with the London meeting last year.

Discontent about Moon's sponsorship of the conference has prompted section chairmen to send a letter to other participants assuring them that the International Cultural Foundation will not interfere in the conference, and that participants will be free to express their own views.

Washington seen

by Colin Norman



But the letter hasn't stilled the discontent and more dropouts can be expected.

● Congress reassembles this week, prepared to do battle with President Ford on oil prices and various other matters. In between the skirmishes, it may get round to passing a bill to re-establish a science policy office in the White House. There has, at least, been some discernable progress on that measure.

Last month, on the eve of the Congressional recess, Representatives Olin Teague and Charles Mosher, respectively the chairman and senior Republican on the House Committee on Science and Technology, jointly introduced a bill which is likely to be passed by the House with little change. The measure is similar in many respects to President Ford's proposal, although it spells out the duties of the office in some detail, and it is prefaced by a

lengthy discourse on the objectives for a national science policy.

The bill would establish a White House Office of Science and Technology Policy (OSTP), headed by a director who would double as the science adviser to the President, and four deputies. Its chief task would be to provide advice to the president on "scientific and technological considerations involved in areas of national concern, including, but not limited to, the economy, national security, health, foreign relations, the environment, and the technological recovery and use of resources. It would also be required to help in the preparation of the federal budget, and "assist the President in providing general leadership and co-ordination of the research and development programs of the federal government". The bill does not state how the office should function within the White House, however, leaving such arrangements for the President to determine.

One item in the bill which was not included in Ford's proposal is the setting up of a Presidential commission, consisting of between 5 and 12 scientists, to carry out a 15-month study of the federal government's science and technology programmes, and to recommend ways in which the overall effort can be improved.

The bill is expected to be passed by the House Science and Technology Committee later this month, and by the full House in October. Meanwhile, the Senate Committees on Labor and Public Welfare, Science and Astronautics, and Commerce, which all have jurisdiction over the bill, will probably hold hearings later this month.

One factor which could upset the timing however, is that Teague was taken ill last month with what was believed to be a stroke, and although he has been discharged from hospital, it is unclear whether his committee schedules will be affected.

● The National Science Foundation (NSF) which has been having severe political problems with critics in Congress, has so far been let off lightly. An amendment designed to give Congress power to veto any individual NSF grant before it is awarded, which was attached to a bill earlier this year by the House has finally been discarded completely. The final version of the bill was passed without the amendment last month and sent to President Ford.

● Finally, the Atomic Safety and Licensing Board has demonstrated a fine sense of irony. It has scheduled a hearing on the proposed demonstration liquid metal fast breeder reactor, which has broken all records for cost overruns, in the US Bankruptcy Courtroom.

correspondence

Spoon bending: an experimental approach

SIR,—We have investigated six young people who claimed the power of bending objects by stroking in the manner demonstrated on television recently by Uri Geller and others. In this report we will call these people A, B, C, D, E, and F. A, B, and F are young girls all aged eleven years. C is a girl of thirteen, while D and E are boys aged ten and eight respectively. All were contacted with the aid of local press and television. A, B and C had received publicity in the local evening papers. Subsequently Dr Pamplin appeared on BBC "Points West" local television news programme when B demonstrated her ability quite convincingly. The parents of D, E and F subsequently contacted Dr Pamplin claiming that their children could also bend cutlery by stroking.

Most of the subjects were first visited in their own homes where they showed their ability in the casual atmosphere of their sitting rooms. A succeeded in bending a weighed and measured rod of mild steel of 3/10ths inch diameter supplied by the experimenters as well as her own cutlery.

All six subjects were subsequently tested in Bath University's psychology laboratory. This laboratory has three large one-way mirrors behind which the experimenter can observe, photograph and take television video tape unseen by the subject. In all cases, except A, one or more observers sat in the laboratory with the subject. In the first four experiments there was a second television camera in the laboratory with the observers. The subject was handed the spoon or rod after its outline had been drawn on a sheet of paper and was allowed to stroke it in the approved manner between forefinger and thumb of one hand and to report what they felt was happening. B reported that the spoon "felt soft" before bending. C stated that it "felt like plaster, then running water". The running water feeling occurred, it was said, just before bending occurred. However, at no time did C bend anything for us while experimenters were watching. The others all succeeded in bending spoons and B bent a rod of mild steel as well.

The aim of the experiment was to obtain a photographic or video taped record of the actual moment of

bending. The observer in the room measured the spoon against its outline at intervals during the session noting the time.

The observers in the room were instructed to deliberately relax their vigilance at intervals after the first twenty minutes. The experimenters were specially alert during these periods and in all cases except C they observed and photographed cheating by the subjects. A put the rod under her foot to bend it; B, E and F used two hands to bend the spoon using considerable muscular power, while D tried to hide his hands under a table to bend a spoon in both hands out of sight of the observer.

We can assert that in no case did we observe a rod or spoon bent other than by palpably normal means. We cannot, of course, conclude that all instances of the so called Geller Effect are due to cheating. However we offer details of our experimental procedure in the hope of helping other experimenters design experiments that can be used when cheating is suspected.

DR BRIAN R. PAMPLIN

MR HARRY COLLINS

Bath University, UK

Presto! Would you believe?

SIR,—In his review of John Taylor's book, "Superminds", Dr C. Evans comments on the ease with which the author seems able to discount the possibility of fraud and deceit on the part of the children involved in purported "psychic" performances. I recently launched an experiment with a newspaper which reports psychic phenomena to prove that persons lacking expertise can easily be deceived by a person using very simple methods—if the predisposition to belief is there. I am a professional conjuror, and needed only modest skills to convince the newspaper that I was indeed possessed of very strong paranormal abilities. It was only necessary to claim genuine powers for the observer to relax completely and believe all.

The need to have a competent conjuror present at performances of this nature is very evident. Otherwise, the observations are of no value scientifically.

JAMES RANDI

51 Lennox Avenue,
Rumson,
New Jersey

Publishing problems

SIR,—I wish to draw your attention to the difficulties which Czechoslovakian scientists seem to be encountering when publishing in international journals.

From a letter which I received from one of our contributors I learn that a paper sent to him arrived in Prague on April 7, 1975, but the author did not receive it until May 23, 1975. Furthermore, he was "commanded out" of Prague on May 24 for two weeks.

I am wondering what these ominous words "commanded out" actually mean. As fellow scientists, we have all been deeply conscious of the treatment received by some of our colleagues in Russia, who have been "frozen out" or even interned.

Let us hope that the Czechoslovakian government will not resort to Soviet methods and will continue to allow their talented scientists to publish in international journals.

JØRGEN CLAUSEN

Neurobiology,
Copenhagen, Denmark

Additive safety

SIR,—We tend to trust Mother Nature, and suspect the organic chemist", says Thomas Jukes mockingly in his vivid defence of synthetic food additives August 7. There is, of course, a reason to trust natural food better than food additives which must be familiar to the author of *Molecules and Evolution*: Organic chemistry happens to be 147 years old; man is at least 1×10^6 years old, and has thus fed for 999,853 years on natural food. The fact that man has survived all these effects of "numerous chemicals (in natural food) that are all more or less toxic" does not prove at all that there will be some fittest to survive the ingestion of at least as many food additives introduced mostly in the past 10-20 years.

Indeed, we do not know whether sodium benzoate has some disagreeable effects within 3 or 10 generations, whereas sugar ("one of the most ancient and universal of food preservatives") has passed the most rigorous toxicological test there is: time. Besides, sugar tastes better.

R. E. HUMBEL
University of Zürich, Switzerland

news and views

STARTING, paradoxically, with invisibly faint electron micrographs of purple membrane patches from *Halobacterium halobium*, Nigel Unwin and Richard Henderson have reconstructed the first distinct electron images of the polypeptide chain packing inside a protein molecule (see page 28 of this issue of *Nature*). This breakthrough at the MRC Laboratory of Molecular Biology in Cambridge is a dramatic culmination of the electron micrographic "crystallography"—or Fourier microscopy—developed there by Aaron Klug and his colleagues over the past decade.

Unwin and Henderson's technique opens up a new frontier in the exploration of the internal structure of proteins—particularly those which build periodic cellular assemblies unsuited for X-ray diffraction crystallography. No one name has yet been devised to identify their method but it can be described as high-resolution, low-dose, under-focus phase-contrast, three-dimensional electron microscopy of thin, unstained, periodic biological specimens.

Drying, staining and electron irradiation can pretty well destroy the molecular order in proteins. Until now significant detail finer than about 20 Å in biological structures has rarely been revealed by electron microscopy even though good microscopes can give 3 Å point-to-point resolution. Unwin and Henderson have mitigated the trauma of dehydration on their specimens by the clever trick of replacing the water with glucose syrup (*J. molec. Biol.*, **94**, 925; 1975). Electron diffraction patterns from their sweetened purple membranes and thin catalase crystals demonstrate that periodic order is preserved out to 3.5 Å spacing, as long as the radiation dose is less than about 0.5 electrons Å⁻². They have obtained micrographs at 7 Å resolution by leaving out the stain which eliminates the usual source of contrast, and by cutting down the electron dose, which raises the noise level well above the signal. Fourier transformation is crucial for correcting, averaging and recombining the invisible microscopic images.

Contrast is produced in the micrograph of the transparent object by underfocusing. But this phase contrast oscillates between positive and negative with increasing spatial frequency, so compensation is essential to recover an undistorted image. The focus is restored using the Fourier processing

Distinct images from invisible micrographs

from D. L. D. Caspar

method previously applied to negatively stained specimens: the computed Fourier transform of the featureless low-dose micrograph—which shows sharp diffraction maxima from the regular lattice—is divided by the modulation transfer function to obtain the correct phases of the object with maximum contrast. The nodes of the transfer function are measured from the optical diffraction pattern of a high-dose micrograph which displays the contrast oscillations although the specimen itself has been incinerated.

At the safe dose for the 7 Å resolution image, only about 25 electrons on the average pass through each 7 Å × 7 Å square of the specimen. Since the difference in the number of electrons forming dark and light regions of the image at maximum phase contrast is less than 1%, the local statistical fluctuations in the micrograph are much larger than the signal. The weak signal from the periodic object is extracted from the noise by averaging over some thousands of unit cells. Evaluation of the corrected Fourier transform at the reciprocal lattice points and retransformation effects this averaging to give an in-focus projected image of a single unit of the real lattice. This is the essence of the optical filtering method: the larger the number of regularly arranged units in the lattice, the sharper the diffraction spots and the weaker the periodic signal in the image which can be distinguished from the noise whose transform extends throughout reciprocal space. The intensity of the spots is actually measured from the electron diffraction pattern of the periodic specimen, but the indispensable phase information is obtained by computation of the compensated Fourier transform of the low-dose, under-focus image.

Tilting the purple membrane lattices provides projected views from different directions which are recombined using the Fourier method of three-dimensional image reconstruction. Correcting for defocusing from large tilted specimens and refining the phase calculations to the same origin for

different views presents special problems which have been resourcefully solved by Unwin and Henderson. The maximum angle of tilt for these micrographs is 57° which leaves about 37% of the 7 Å sphere in reciprocal space unsampled. Fortunately, the amplitudes of the Fourier components in the missing regions are small. Thus, their 7 Å resolution map provides an accurate representation of the α -helical rods extending roughly perpendicular to the plane of the membrane, although the short connecting segments parallel to the surface are not yet seen. This remarkable three-dimensional picture illuminates both the objectives and the methods of structural biology.

In order to see inside protein molecules sharply at high resolution, Unwin and Henderson record very faint, out-of-focus micrographs of large periodic arrays at relatively low magnification. This "coincidence of contraries" reflects the antithesis of "distinct images and clear conceptions" recognised by S. T. Coleridge in a more epistemological context. The concepts for dealing with periodic images in real space have been most coherently formulated in reciprocal space in the 60 years since W. H. Bragg first pointed out the utility of Fourier synthesis for crystal structure analysis. Fourier transformation is not the only way to recover information about three-dimensional structure from electron micrographs but it has been, so far, the constructive way.

The development of Fourier microscopy of biological structures in Cambridge recapitulates in many ways the history of X-ray crystallography started there by W. L. Bragg in 1913 and, more particularly, protein crystallography begun there by J. D. Bernal and Dorothy Crowfoot Hodgkin in 1934. By the early 1950s Max Perutz and W. L. Bragg were mapping the outline of proteins by tracing the molecular transform and by using a kind of negative staining for X-ray diffraction; the internal structure of proteins became visible with X-rays in 1953 when Perutz successfully applied the isomorphous replacement method to solve the phase problem.

Electron micrographic crystallography was begun in the mid-1960s by Klug and his colleagues using optical diffraction and by 1968 it had become Fourier microscopy using computation of Fourier transforms of digitised images for the reconstruction of three-dimensional structures. There

is no "phase problem" in this microscopy since the phase information is contained in the image—but there was a "specimen problem" since the images, at best, showed little more than the shadow of the stain encasing the biological molecules. Unwin and Henderson have now introduced an ingenious but simple technique for solving the specimen problem which makes it possible to see inside molecules with electrons, perhaps to the ultimate limit of resolution of the microscope itself.

Any thin periodic structure with large ordered domains that can survive the extreme environment inside an electron microscope is a suitable object for imaging by this method. Their approach will be very useful for analysing the structure of molecules, such as lipids and some proteins, that form thin crystals difficult to study by X-ray diffraction. The unique power of the method for determining the structure of molecules that build cellular "surface crystals" is beautifully demon-

strated by Unwin and Henderson's image reconstruction of the purple membrane.

The purple membrane was isolated seven years ago in Walther Stoeckenius' laboratory as a pigmented by-product of studies on some other curious structures from this halophilic bacterium; it has now become an object of central importance in membrane biology. Photons absorbed by the chromophore, retinal, pump protons across this specialised membrane assembly. Analysis of the operation of this system is clarifying basic mechanisms in vision, photosynthesis and oxidative phosphorylation. Unwin and Henderson's image of the structure is comparably informative. The interlocking of the X-helical segments of the purple membrane protein, as in Crick's coiled-coil, and the fitting of the lipid bilayer in the interstices of the protein lattice illustrate key aspects of the structure of structural proteins and the organisation of membranes.

site is even more significant since it was there that geothermal power was first produced successfully (in 1904) when steam was piped to low pressure turbines driving small electrical generators. The dry (superheated) steam power stations at Larderello still account for about 40% of the world's geothermal power output, although there is now also a wet steam power station complex at Wairakei in New Zealand, a dry steam system at The Geysers in California and smaller stations in Japan, Iceland, Mexico and the USSR. The total geothermal power capacity installed throughout the world is now about 1,000 MW, which is still an insignificant proportion of the total power from all sources. In addition to power generation, however, geothermal steam is also used directly in various parts of the world for paper making, sewage treatment, potash mining, district space heating, growing greenhouse crops in cold climates and for many other purposes which can be effected close to the geothermal sources themselves.

The variety of uses for geothermal heat is impressive even without adding proposals in the pipeline. But as Morton C Smith of the Los Alamos Scientific Laboratory pointed out last year at a meeting of the American Institute of Physics, the development of types of sources currently in use, especially for power generation, poses numerous as yet unresolved problems. For one thing, dry steam reservoirs such as those at Larderello and The Geysers seem to be extremely rare. There are fewer than a dozen known 'vapour dominated' geothermal fields of possible commercial size throughout the world, the largest of which would be capable of development to no more than 3,000 MW. It is possible, of course, that there are many dry steam sources which remain undiscovered because they have no surface indicators; but if so, the chances of finding them quickly are small because remote sensing systems capable of detecting subterranean steam are still poorly developed. Moreover, there are many scientific and technical unknowns. Is a dry steam reservoir a wasting or self-renewing asset? What are the interactions between heat flow, fluid flow, mass transport and tectonic stress changes? How can minerals (silica, for example) deposited from the steam be prevented from clogging up the works and volatiles (for example, hydrogen sulphide and boron and arsenic compounds) be prevented from causing pollution? Notwithstanding operating experience to date, none of these questions has been completely answered.

Natural geothermal systems in which the fluid is mainly hot water are much more common than those containing

Towards universal geothermal power

from Peter J. Smith

"WE are, with regard to this problem of utilizing the Earth's internal heat, very much in the position of an eighteenth century 'outcropper' quarrying for coal in the hillsides of Yorkshire. Noting the dip of the strata, he could quite well reason that far below the surface there must be immense reserves of coal, which on the one hand he did not need to exploit because the hillside quarry provided for all his immediate needs, and which on the other he could see no possibility of exploiting at a profit."

These words were spoken by John L. Hodgson at the British Association meeting in Leeds 48 years ago this week; but a similar statement could almost have been made today. The only difference is that the more generalised mid-20th century version of the 'hillside quarry'—cheap and abundant fossil fuel—is no longer with us. It disappeared so recently, however, that until now there has been little incentive to tap the "vast potentialities" of geothermal energy that Hodgson dreamt of.

Indeed, the whole subject of the Earth's internal heat has developed remarkably slowly from its origins in the 17th century. The earliest known reference to high temperatures within the Earth is that by Morin (*Nova Mundi Sublunaris Anatomia*, Paris, 1619) who found the air becoming warmer as he penetrated deeper into a

group of Hungarian mines. Some 50 years later Robert Boyle recorded (others') eyewitness accounts of temperature increases in mines and discussed the source and transport of underground heat, concluding that heat of chemical origin from the Earth's deep interior rises by conduction and the movement of material to produce the observed near-surface increase of temperature with depth.

From 1868 to 1883 a committee of the British Association pursued the subject vigorously and systematically and, having finally appreciated that the determination of heat flow requires the measurement of thermal conductivity as well as temperature gradient, obtained a mean heat flow of $1.3 \mu\text{calorie cm}^{-2} \text{ s}^{-1}$, a value only slightly lower than today's average based on many more data.

But although 'academic' studies of heat flow are now beginning to contribute to the search for sources of geothermal power, the practical use of the Earth's internal heat (which has a much longer history) apparently proceeded quite independently, largely on the basis of chance discoveries of particularly conspicuous and high local concentrations of near-surface heat.

On the industrial front, the Italians have been extracting boric acid from natural steam jets at Larderello in Tuscany since the late 18th century. Historically, however, the Larderello

predominantly dry steam; but the problems of power evaluation, development and production here are even greater, largely because of dissolved minerals. The natural pressures in most hydrothermal reservoirs are too low to maintain artesian flow, so the water must be pumped to the surface. But the hot brines rapidly destroy drilling equipment, borehole casings, pumps, valves and the rest of the hardware required to bring the fluid to the surface and extract heat from it. And once the heat is extracted the residual highly-concentrated brines then pose waste (thermal and chemical) disposal problems. The removal of large quantities of subsurface fluid can also lead to extensive subsidence. According to Smith, therefore, "the widespread exploitation of a large and readily available geothermal resource will evidently be delayed for some years until economic solutions to [these problems] have been demonstrated".

Where, then, does the long-term future of geothermal energy exploitation lie? The answer favoured by the Los Alamos group and currently being investigated by them is the extraction of heat from dry rock. Even in steam and water reservoirs well over half of the thermal energy resides in the rock, and rocks at depth are hot even when fluid is absent. In principle, therefore, it should be possible to extract geothermal heat anywhere in the world as long as sufficiently high temperatures are reached at depths to which drilling is economic.

Although it is seldom, if ever, acknowledged, the simplest way of achieving this dream was described in some detail by Hodgson in his talk in 1927 when he proposed an artificial version of the hydrothermal system that nature itself rarely provides. In other words, he suggested that cold water should be passed through the hot rock at depth and then recovered in the form of hot water or steam. This is almost exactly the method now adopted by the Los Alamos group with the difference that, whereas Hodgson envisaged a closed pipe circulation, the modern version has the injected water making direct physical contact with the hot rock and rising through a second borehole. An obvious unknown in either system, however, is whether there is an adequate area of contact at depth to achieve sufficient rock-to-water heat transfer. Hodgson's solution was to increase the length of his pipe at the bottom of the borehole if necessary; the modern idea is to fracture the rock at depth.

But whereas Hodgson's proposal was destined to remain unrealised, the Los Alamos suggestion is actually being put to the test at a site in New Mexico. According to Laughlin (*Geotimes*, 20,

March 1975), the main hole has already been drilled to a depth of 2,929 m in impermeable rock where the bottom-hole temperature is 196 °C. Hydraulic fracturing of the surrounding rock was successfully carried out at 1,981 m and will be repeated at the bottom. A second, somewhat shallower, hole is then to be drilled into the lower fracture zone to act as the hot fluid ascent route.

If the dry rock experiment in New Mexico proves scientifically and technically successful and an economically viable geothermal heat extraction system results, the way would clearly be open to a staggering increase in the use of this widely available and relatively pollution-free energy resource. Presumably it is still far too early to say when, or even whether, this ideal state will ever be reached. In the meantime, however, it must be evident that, other things being equal, both science and economics would benefit from application to areas where the heat flow is relatively high and the thermal energy is close to the surface.

The high heat flow anomaly in the Gulf of California, whose discovery is reported by Lawver *et al.* on page 23 of this issue of *Nature*, fulfils both of these criteria, having a remarkably high maximum value of 30 $\mu\text{calorie cm}^{-2} \text{ s}^{-1}$ (which compares with 4.5 $\mu\text{calorie cm}^{-2} \text{ s}^{-1}$ at the New Mexico site) and coming from a basaltic intrusion within 100 m of the surface. Admittedly the heat source is not continental; nor is it entirely clear to what extent, if any, hydrothermal fluids are involved. What is certain, however, is that Lawver and

his colleagues have discovered one of the Earth's major geothermal resources and that it is in just such areas that the real case for geothermal power will be fought. □

X-ray sources and the ionosphere

from L. J. C. Woolliscroft

THE lower ionosphere is a complex region of the upper atmosphere, with several ionisation mechanisms and high enough neutral species densities to cause many ion and electron reactions with the weakly ionised plasma. The production of ionisation by non-solar sources has been calculated by, for example, Velinov (*J. atmos. terr. Phys.*, **30**, 1891; 1968) for the influence of galactic cosmic radiation on the bottom of the D-region. More recently other non-solar objects have been invoked as sources of ionisation in certain conditions.

The propagation of low frequency (LF) and very low frequency (VLF) radio waves from extremely stable transmitters such as those used to broadcast atomic time allows the bottom of the ionosphere to be monitored continuously. Small changes in the phase and amplitude of the received LF and VLF signals from distant transmitters are used and some of these have been associated with the meridian transit of a strong X-ray source. Edwards *et al.* (*Nature*, **222**, 1053; 1969) found a nocturnal VLF effect from Scorpius XR-1 and the same source was found to give an effect at LF by Ananthakrishnan and Ramnathan (*Nature*, **223**, 488; 1969). Later positive results have included other celestial X-ray sources and also flare effects in Sco XR-1.

That these radio propagation effects are due to X-ray sources was challenged by Poppoff and Whitten (*Nature*, **224**, 1187; 1969) who showed that the ionisation production rate was at least an order of magnitude lower than that due to other mechanisms, especially that of Lyman α radiation ionising nitric oxide. A difficulty exists, however, in calculating the production of ionisation by Lyman α because the concentration of NO is somewhat uncertain.

A further complication comes from the masking effect of precipitating electrons producing ionisation at other than low latitudes which may account for some of the negative results such as those of Burgess and Jones (*Nature*, **224**, 680; 1969).

In this issue Karszenboum and Gagliardini (page 34) have calculated the production rate of ionisation due to several galactic X-ray sources using more recent spectra which extend to



A hundred years ago

PROFESSOR PALMIERI has discovered a new instrument which he calls a "diagometer," and which is constructed for the rapid examination of oils and textures by means of electricity. What the apparatus will do, Prof. Palmieri details thus:—1. It will show the quality of olive oil. 2. It will distinguish olive oil from seed oil. 3. It will indicate whether olive oil, although of the best appearance, has been mixed with seed oil. 4. It will show the quality of seed oils. 5. Finally, it will indicate the presence of cotton in silken or woollen textures. The professor has been complimented for this invention by the Chamber of Arts and Commerce at Naples, who have published a full description of the apparatus, with instructions for use. from *Nature*, **12**, 427; September 9, 1875

higher energies and new data for the photoionisation yields. They compare their production rates for X-ray sources with the nocturnal production by other mechanisms and conclude that at least Sco XR-1 and probably some others could be significant at heights around 80 km.

The daytime production has to compete with solar X rays and ultraviolet, making measurements less easy. Ramanamurty *et al.* (*J. atmos. terr. Phys.*, **32**, 1721; 1970) found an amplitude variation over the LF path from Tashkent to Delhi at the time of an X-ray flare from Sco XR-1 and argued that this was theoretically possible under favourable geometry conditions with certain values for the NO concentration.

If the electron density of the D-region is enhanced by an X-ray source then the conductivity will also increase altering the pattern of ionospheric currents and thus the magnetic field. Murty and Yacob (*Planet. Space Sci.*, **22**, 1583; 1974) have studied the average sidereal time variation of the nocturnal geomagnetic horizontal component and found perturbations at the times of transit of some of the stronger X-ray sources. There is some doubt about their results since the magnitude of the perturbation does not correlate with the strength of the source as one would expect from a simple mechanism, indicating perhaps contributions from other effects.

The first daytime perturbation in the magnetic field is reported by Sastri and Murthy on page 35 of this issue, using magnetometer data during the same Sco XR-1 flare as Ramanamurty *et al.* Unfortunately a small solar X-ray flare occurred 33 min before the magnetic field perturbation, but Sastri and Murthy argue that this was both too weak and too early to cause the effect. The detailed mechanism for these geomagnetic effects remains uncertain but will probably involve a pattern of ionospheric current similar to that at the time of a solar flare.

The use of this ionospheric technique to study other astronomical sources such as γ -ray bursts is being studied by Mongain and Baird (paper A.2.7 presented at Cospar, Varna, June 1975). They argue that for the detection of γ bursts the ionospheric recombination time is very important and the best time may not be at night when the recombination time is short compared with the burst, but during the day. The analysis of their data is currently proceeding but they feel that as yet the work will tell us more about the ionosphere than about astronomy. The recently reported strong transient X-ray source A0621-00 discovered by the satellite Ariel V may also serve as a test for many of the theories. □

Primary, secondary, tertiary

from E. G. Richards

WHAT is the nature of the intercis-tronic regions in *Escherichia coli*? Platt and Yanofsky (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2399; 1975), have recently provided us with an answer in the case of the junction between the B and A cistrons of the *trp* operons. Making use of the high yield of the distal end of the corresponding messenger RNA from the *trp* Δ LD102 mutant and the fact that DNA from phage λ trpB27 and ϕ 80trpA37 carries sequences complementary to the end of the B and the start of the A cistron (and hence will hybridise with the messenger and protect it from nuclease attack in this region), they were able to isolate a fragment of 32 P-labelled RNA about 200 bases long. This was fingerprinted and a comparison of the oligonucleotides produced with the known sequence of the two proteins enabled them to determine most of the sequence. Any doubts about the part between the two translated regions were resolved by fingerprinting and sequencing the shorter polynucleotides produced by binding the longer piece to ribosomes to form the initiation complex of the A cistron and trimming the ends with nucleotides. The final result is of no little interest in that the termination signal for the B protein is found to be a single UGA codon which is out of phase with the AUG initiation triplet for the A protein; indeed, the final A of the termination codon is the initial A of the initiation codon, so that there are only two bases which are not translated. A consequence of this is that if the initiation recognition site of the A protein involves more than this UGAUG sequence it must also be translated as part of the B or A cistron. The authors point out that this and other known instances of dual function in mRNA provide some rationalisation for the degeneracy of the genetic code. They also note that close to the end of the B cistron and within the A cistron ribosomal binding site is a sequence GAGGGG which could bind to the CUCCUU sequence at the 5' end of the 16S rRNA; an interaction between such regions has been proposed by others.

Platt and Yanofsky do not speculate on possible secondary structures of their messenger fragment but such cogitations have been taken up by Pipas and McMahon (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2017; 1975) albeit for tRNA sequences rather than RNA. The consistency of the clover leaf structure with all known tRNA sequences as well as with recent X-ray

crystallographic investigations has probably convinced all but the most hardened sceptic of its reality. Nevertheless it is still valid to ask if the clover leaf structure is the structure with minimum free energy. Several authors have published descriptions of computer programs that will list all possible base pairing schemes compatible with a given sequence and other workers have provided, on the basis of work with model compounds, formulae for assessing the free energy of formation of secondary structures (from the random coil) in terms of contributions from various features such as loops, bulges and helical regions. Pipas and McMahon have put these two notions together and written a program that gives the free energy of all possible structures. They begin, as have others before them, by getting the computer to produce a list of all possible helical regions containing at least three base pairs. GU pairs are allowed but not at the end of a helical region; the hairpin loop must contain at least three bases and there are one or two other restrictions of a similar nature. The next step is to decide whether a given pair of helices are compatible or not, that is, whether they co-exist in the same structure. Clearly two helices that involve the same bases are incompatible and the authors also assume that if the bases in the hairpin loop of one helix participate in the formation of another helix, they only do so by pairing with other bases in this same loop. This latter restriction means that only structures resembling clover leaves are considered and more complicated patterns of folding (which cannot be drawn on a sheet of paper without the chain crossing itself) are not considered. This is probably the greatest weakness in their results but was required at the present state of play by the fact that the data for assessing the free energy of these more complicated structures are at present unknown. The next stage in the calculation was to enumerate one by one all the possible sets of compatible helices and then to work out the free energy of each.

Sixty-two tRNA sequences were presented to this program on a CDC6500 computer and all were analysed in less than 3 minutes. It was thus found that for 32 of these sequences, the clover leaf had the minimum free energy. Of the remainder, the minimum free energy structure of 13 corresponded to clover leaves with one or other (usually the DiHU) of the stems undone, and in five sequences the conventional acceptor stem contained non-standard base pairs which led to its elimination by the rules described above. This left 12 sequences which gave more extended structures as the most stable,

but their free energy was within 5 kcalorie of that of the clover leaf. Several explanations are available for the anomalies: the thermodynamic data on which the energy assessments are based may be faulty in some way or the native clover leaf form may be trapped in a metastable state; but the authors incline to the notion that tertiary interactions may tip the balance in favour of the clover leaf, and in the worst case only 5 kcalorie would be required.

The experimental elucidation of such tertiary interactions by NMR techniques is the subject of an interesting paper by Reid *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2049; 1975). They determined the NMR spectrum of several highly purified species of tRNA in the -9 to -15 p.p.m. region. The -11 to -15 p.p.m. region gives a signal believed to emanate from the hydrogen-bonded ring NH protons and proportional to the number of Watson-Crick base pairs. Nevertheless it has been suspected that this low field region should contain resonances due to other sorts of base pair and to tertiary interactions. Their first interesting observation was that tRNA^{Val}, tRNA^{Arg} and tRNA^{Phe} all from *E. coli* gave a resonance corresponding to two protons at about -10.5 p.p.m., whereas tRNA^{Asp} from yeast gave a signal corresponding to about 10 protons in this region. The three *E. coli* sequences all indicate one GU base pair somewhere in their clover leaf structures while the yeast sequence indicates no less than three GU pairs and a GΨ pair as well. Since a GU pair involves two ring NH protons hydrogen bonded to oxygen atoms, the authors tentatively assign the -10.5 p.p.m. resonance to GU pairs.

The next point concerns a single proton resonance at -14.9 p.p.m. observed with these same three *E. coli* species but absent in several yeast tRNA spectra. For these *E. coli* species ring current shifts were not expected to result in any resonance below -14.5 p.p.m. Now the *E. coli* sequences all have s⁴U in position 8, whereas yeast tRNA^{Phe} has an unmodified U in this position which the X-ray diffraction data suggest is hydrogen bonded to A14. This suggested the attractive hypothesis that the -14.9 p.p.m. resonance corresponded to a S⁴U8-A14 base pair. This was neatly confirmed in the case of *E. coli* tRNA^{Val} by removing the sulphur by the action of cyanogen bromide. When this had been done, the tRNA was still active in aminoacylation (and hence presumably still had the native tertiary structure) but the -14.9 p.p.m. resonance had gone.

Other resonances in the -11 to -15 p.p.m. region probably remain to be

defined and assigned, because in the case of *E. coli* tRNA^{Val}, the integrated intensity from this region corresponds to 26±3 protons. One of these is, no doubt, the s⁴U-A tertiary interaction and 20 more no doubt correspond to the 20 Watson-Crick base pairs in the structure, but this leaves several more unaccounted for. The authors also suggest it likely that resonances corresponding to four protons in the -9 to -10 p.p.m. region in the case of *E. coli* tRNA^{Val} should be assigned to amino protons hydrogen bonded to ring nitrogens. Several such hydrogen bonds appear in the tertiary interactions discerned in yeast tRNA^{Phe} and *E. coli* tRNA^{Val} has corresponding bases in corresponding positions of the sequence.

Clearly more work needs to be done to confirm some of these tentative assignments and to elucidate others but this technique is reaching the point where it can provide information concerning the three dimensional structure of nucleic acids which is almost on a par with that obtained from X-ray crystallography. □

Ectoparasite provides cercarial model

from F. E. G. Cox

DIGENETIC flukes typically use two hosts during their life cycle, a mollusc and a vertebrate. The larval stage infective to the vertebrate is the cercaria which passes out from the snail and often has a short free-swimming existence before it gains access to its final host. Cercarial activity and infectivity have been studied in economically important species such as *Schistosoma mansoni* and *Fasciola hepatica* but it has been difficult to obtain much quantitative information from these studies because of problems such as having to dissect the final host to find out how many cercariae have actually established themselves. Because these problems are inherent in dealing with any internal parasite there has been no particular interest in developing alternative laboratory models and the study of the digeneans has therefore tended to suffer.

A convenient model is now available and it is an ectoparasite so easy to maintain that it will obviously become very important in the future. This fluke is *Transversotrema patialensis* and it lives under the scales of many freshwater fish including *Brachydanio rerio*, the Zebra Danio, which is sold

by many shops stocking tropical fish. The snail host is *Melanoides tuberculata* which is also available from aquarists. When infected fish are put in a tank containing uninfected snails the life cycle maintains itself indefinitely and the tank provides a convenient source of every stage required for experimental investigations. It is also easy to see how many adult flukes have become established simply by looking at the fish alive.

Transversotrema patialensis is therefore an ideal subject for the study of cercarial survival, activity and infectivity. Anderson and Whitfield (*Parasitology*, **70**, 295; 1975) have found that cercariae survive for a maximum of 44 h and that infectivity is restricted to half this time. As these cercariae do not feed they can live only as long as their energy reserves persist; Anderson and Whitfield have produced theoretical models relating infectivity to activity and activity to the amount of glycogen available and have found good correlations between their models and their experimental observations. They used electron microscopy as well as the more usual histochemical techniques in order to observe the depletion of glycogen in the cercariae.

Because the period of infectivity is so short the cercariae must locate, identify and attach to an appropriate host as quickly as possible. In the logical follow-up to the first study Whitfield, Anderson and Moloney (*Parasitology*, **70**, 311; 1975) were able to conclude that the location of the fish is a random process but that essential information regarding the suitability of the host is probably obtained by the use of receptors on the tail of the cercaria. Scanning and transmission electron microscope studies show that these receptors are unique but similar to types found in monogeneans, also ectoparasites, and which are thought to be concerned with chemoreception. After recognition of the host, attachment is accomplished first by means of adhesive pads, also on the tail, and then by the ventral sucker on the body after which the tail is lost and the fluke attains its final position under a scale.

These experiments open up a whole new field of investigation into the biology of flukes because the number of flukes that have actually established themselves can be counted without the need for killing and dissecting the host. These two papers are only a beginning but the standard set should ensure that further studies will also be based on critical experimental observations coupled with the use of techniques such as electron microscopy and mathematical analysis when necessary.

Solar oblateness

from David W. Hughes

THE Sun is thought to be flattened. Dicke and Goldenberg (*Astrophys. J. Suppl.*, **27**, 131; 1974) gave $(4.51 \pm 0.34) \times 10^{-5}$ as the value for this oblateness which means that the polar radius is 31.4 km smaller than the equatorial radius. Not much considering the mean solar radius is 695,997 km. Now it is clear that a rapidly rotating solar core with a period of between 0.5 and 2 d could provide an explanation for this flattening; but what is not clear is why the core should rotate so rapidly. This problem has been approached theoretically in a recent article by Schatten (*Astrophys. Space Sci.*, **34**, 467; 1975).

Obviously there must be some interaction between the quickly rotating core and the slowly rotating photosphere. Because the solar interior contains essentially all the mass and the angular momentum the photosphere would be spun up to the angular velocity of the interior unless there were some braking force acting on it. Dicke suggested that it is the accelerating torque exerted on the solar wind by the magnetic fields locked in the photosphere which provide this brake. In his first approach to the problem Schatten assumes that the rate of change of angular velocity with time is negligible and then finds that the angular velocity of the solar core is simply obtained by dividing the solar wind's torque, τ_{sw} , by the rate of change of the solar moment of inertia with time dI/dt . This moment of inertia varies because the nuclear reaction in which hydrogen is converted into helium reduces the number of ionised particles in the solar interior. To conserve the number of particles per unit volume and the pressure balance the core has to contract. Taking τ_{sw} as -8×10^{30} dyne cm, calculations of dI/dt as a function of r , the distance from the centre of the Sun, gives a rotation period of 17.5 h for $r < 117,000$ km and $25.4 (r/R_{\odot})^2$ days for $r > 117,000$ km.

In Schatten's second approach the square of the angular velocity of the core is found to equal the mean power loss due to solar activity, P_{sa} , divided by $0.5 dI/dt$. As the core contracts the rotation power is liberated through the magnetic fields in sunspots. The energy lost in a solar cycle is calculated to be 10^{35} erg, equivalent to 2×10^{27} erg s^{-1} . However some of the spot energy might not get away from the Sun and Schatten, considering flares, gets a more dependable value of 10^{26} erg s^{-1} . These two values give a core rotation period of between 1 and 4 d. Third, the core rotation period must equal P_{sa}/τ_{sw} which gives periods of 0.5 to 5 d. These theoretical values of core

rotational velocity, based upon simple principles of conservation of angular momentum, energy and torque flow from the Sun, give a total oblateness of 3.4×10^{-5} for the photosphere which is in close agreement with the observed value of 4.5×10^{-5} .

Three interesting problems still remain. How does the solar core maintain its rapid rotation, bearing in mind that it is a highly conducting plasma? Also what effect does this oblateness have on celestial mechanics? The value obtained would produce a 4.05 s of arc per century advance in the perihelion of Mercury. This opens up the question of the accuracy of the general relativistic tensor theory of gravitation in comparison, say, with the scalar tensor theory. Third, can the rapid rotation of the core produce a lower central temperature in the Sun and thus help explain why the neutrino flux is less than expected?

It must be mentioned, however, that not everyone agrees with the oblateness values obtained by Dicke and Goldenberg. Hill and Stebbins in a paper scheduled for the September edition of *The Astrophysical Journal* discuss observations they have made at the University of Arizona's observatory in the Santa Catalina Mountains. The value they obtained for the oblateness is $(8.6 \pm 5.9) \times 10^{-6}$ which indicates that the Sun's shape is indistinguishable from that of a sphere. □

Nitrogen in synthetic diamond

from John Walker

DIAMONDS might be a girl's best friend, but in many respects they are very puzzling to scientists. One of the most intriguing problems concerns nitrogen, which is a very common impurity in diamond, and which has recently been studied in synthetic crystals by some Soviet scientists (Yu. A. Klyuev, V. I. Nepsha, and A. M. Naletov, *Sov. Phys. Solid State*, **16**, 2118; 1975). Nitrogen occurs in aggregated form in most natural diamonds, but in a few rare crystals the nitrogen atoms are isolated, each one replacing a single carbon atom. These crystals can be detected because the nitrogen is paramagnetic—it can be observed in magnetic resonance experiments.

Surprisingly enough, synthetic diamonds usually contain isolated paramagnetic nitrogen rather than clusters. To investigate this difference, and to check the earlier magnetic resonance work on natural diamond, Klyuev *et al.* have grown synthetic diamonds doped with the rare nitrogen isotope ^{15}N . This isotope has a nuclear spin of one-half, which gives rise to a magnetic

HLA histocompatibility system

In the article "Histocompatibility testing international" which appeared in the News and Views Section last week, the designation of the HLA system was changed during editing to HL-A. The WHO nomenclature committee mentioned in the report did in fact recommend that the whole region should now be designated HLA.

resonance spectrum somewhat different from the ^{14}N which predominates in natural diamond and has a spin of unity. The Russian workers were able to confirm the earlier work, allowing for the different nuclear spin.

Nitrogen in diamond can also be detected using infrared spectroscopy. The absorption spectra of the ^{15}N diamonds were very similar to those of ^{14}N crystals, except that a peak at $1,135 \text{ cm}^{-1}$ in the latter had shifted to $1,120 \text{ cm}^{-1}$. This was accounted for by the different masses of the two isotopes. A second peak, at $1,282 \text{ cm}^{-1}$, which can be caused by nitrogen clusters, was relatively strong in the doped crystals, irrespective of the isotope used. Undoped diamonds were also found to contain paramagnetic nitrogen, presumably as a result of contamination from the atmosphere, but had a relatively weak $1,282 \text{ cm}^{-1}$ peak. This suggests that nitrogen clusters are present as a minor species in doped synthetic crystals.

The most likely cause of this difference in nitrogen's behaviour in natural and synthetic diamonds is that different pressures and temperatures are used in the two crystallisation processes. Laboratory studies by the same authors (Yu. A. Klyuev, Yu. A. Dudenkov, and V. I. Nepsha, *Geo. chem. Int.*, **7**, 781; 1973) have shown that relatively high pressures and low temperatures during the synthesis process create cubic crystals which contain a lot of dispersed nitrogen, but few aggregates. Lower pressures and higher temperatures create octahedral crystals with a lot of nitrogen clusters, very similar to natural diamonds. What is not clear is whether the aggregates are formed directly during the crystallisation process, or whether they grow afterwards, in the high pressures and temperatures needed for synthesis. The authors favour the latter hypothesis, but further work is needed to decide the issue. The fact that synthetic diamond can now be doped with a specific isotope indicates how well the process can be controlled. This has implications not only for diamond physicists, but also in the realm of new applications. □

review article

Electrons in glass

Nevill Mott*

Our understanding of the behaviour of electrons in glasses, liquids and non-crystalline materials generally is decades behind the detailed theory available for crystals such as silicon and germanium. This article describes the advances made in the past fifteen years. It includes the work of the Leningrad school on amorphous semiconductors, the reason why they cannot usually be doped, the concept of "variable-range" hopping and the Cohen-Fritzsche-Ovshinsky and other models for the conduction band, and the reason why glass can be transparent. A crucial experiment, that of two-dimensional conduction in an inversion layer at a Si/SiO₂ interface, is described. Particular emphasis is given to concepts about which there is a disagreement and indeed controversy, such as the "mobility edge", the "minimum metallic conductivity" and the interpretation to be given to the ovonic threshold switch.

EXTRACTION of metals and the manufacturing of glass are two of our oldest technologies; metals are important because they are strong and ductile and glass because it is transparent. And yet it is remarkable that a serious attempt to understand the ductility of metals in terms of the movement of atoms came later than, for instance, the discovery of the neutron, though now it is fully achieved; on the other hand the transparency of glass and equally the properties of some glasses as semiconductors are still not properly understood, and cannot yet be calculated in the detailed way that the application of quantum mechanics has made possible for crystalline materials such as silicon. At the same time there has in the past few years been a surge of interest in the electrical properties of glasses and of non-crystalline—that is to say amorphous—materials generally; this article describes what has been happening, and why.

Interest arose from several sources. The photocopying process known as xerography makes use of photoconduction in amorphous selenium and led to very extensive work on that material in the Rochester Laboratories of the Xerox Corporation. The development by Ovshinsky and coworkers of an electronic switch based on thin glassy films of a mixture of selenium, tellurium, arsenic, germanium (STAG glasses) started a lively controversy which is not yet over. Work in Leningrad, Prague and Bucharest pioneered some aspects of the subject; in particular Kolomiets (for review see ref. 1) in the Ioffe Institute at Leningrad showed that the chalcogenide (that is, based on S, Se, Te) glasses, such as As₂Te₃ and the STAG glasses, behaved like intrinsic semiconductors and with a resistivity which, markedly unlike crystals, could not be much lowered by doping.

On the theoretical side the work of Ziman² showed that electrical conduction in one class of non-crystalline material, liquid metals, could be very simply understood. Also in 1958 P. W. Anderson's difficult paper³ on the absence of diffusion in certain random lattices, which he himself says is "often quoted but rarely read", introduced the idea of localisation: whereas an extra electron introduced into a crystal, with energy (in the jargon of the subject) "in the conduction band", can move quite freely but may be trapped by impurities, the mere absence of crystalline order can lead to a range of energies for which

an electron is trapped even in the absence of impurities. Anderson's paper proved controversial and—like many new ideas—took some ten years and many international conferences to establish itself.

With this introduction I shall return to the fact that glass is transparent. In crystalline materials our understanding of the difference between transparent non-metals and opaque metals goes back to the first application of quantum mechanics to solids by Bloch, Peierls and Wilson in the early 1930s. They treated electrons as freely moving, and described by wave functions with well defined wavelengths; for certain wavelengths Bragg reflection from the lattice takes place, leading to the well-known energy gaps and to the concept of full and empty bands for the allowed energies of the electrons. A transparent non-metal is a substance in which a full band is separated from an empty band by an "energy gap", with energy greater than the quantum of visible light. But this description depends essentially on the crystallinity of the material; there are no sharp Bragg reflections from glasses for electrons, any more than there are for X rays. In fact, when theorists round about 1968 first noticed that there was a problem here, one paper at an international conference was entitled "How can there be an amorphous semiconductor?" One could ask too "How can there be a transparent glass?", and this is part of the same question.

A first clue came from the discovery by Kolomiets¹ that, as already stated the low conductivity of the chalcogenide glasses is not increased even by very strong doping. The same is true of amorphous films of silicon which can be deposited by various methods. When the film recrystallises, but not before, the impurities will act as donors and will cause the conductivity to shoot up. The most likely explanation is the following⁴. In crystalline silicon, which has the diamond structure with each of the four valence electrons taken up in a covalent bond, an atom of phosphorus (with five electrons) is incorporated substitutionally, four electrons being used in bonds and the fifth being very weakly bound, so that at room temperature it is free to move and can contribute to a current. In a glass or in amorphous silicon, on the other hand, the structure will normally accommodate itself so that any atom has the right number of neighbours to accommodate all the electrons in bonds; phosphorous if pentavalent should have five, or if trivalent three. The absence of freely or loosely bound mobile electrons is why any

*Address: Cavendish Laboratory, Cambridge CB3 0HE, UK.

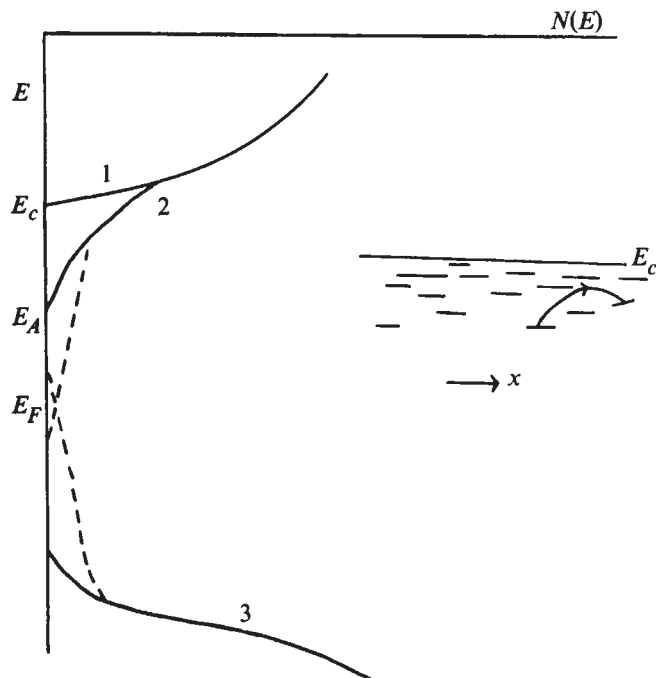


Fig. 1 Density of states $N(E)$ in an amorphous semiconductor plotted against energy. 1, Shows the form of $N(E)$ for the conduction band of a crystalline semiconductor; 2, the probable true form for the amorphous material; 3, the valence band and the dotted lines the "tails" as proposed by Cohen, Fritzsche and Ovshinsky. E_F is the Fermi energy and E_c the mobility edge. On the right the horizontal lines show energy levels in the traps (localised states), x denoting the position on space occupied by the traps. The line with the arrow shows a typical hopping process.

glass is transparent. Colour in stained glasses is due to transition metal ions, where the inner electrons do not necessarily form covalent bonds and therefore are available for absorbing or scattering visible light.

A description of this kind is a long way from that usual among physicists for, say, crystalline SiO_2 or silicon, where all electrons are treated as free and the "energy gap" is a consequence of Bragg reflection. To treat the electrons as being stuck in bonds and to go no further seems a reversion to pre-quantum mechanical thought⁵. At present very active endeavours are in progress to bring a proper theoretical treatment to this model. In spite of a great deal of success, this work has not yet answered what is probably the most important question for glassy semiconductors, namely, what is the density of electronic states near the bottom of the conduction band? This is where electrons with thermal energies will be. For a crystal this density behaves like $\sqrt{E}$, where E is the energy; in a glass there should undoubtedly be a "tail", due to disorder, as suggested in Fig. 1. Those who feel that a gap is inconsistent with a non-crystalline structure have thought that the tail should extend an indefinite distance into the gap (the dotted line in Fig. 1) and should overlap a tail from the valence band; this was indeed proposed by Cohen, Fritzsche and Ovshinsky⁶ (the CFO model). But it now seems almost certain from observations that the "tails" are for all practical purposes limited; unless the glass has structural defects such as missing atoms, the band has a lower edge (E_A in Fig. 1). The states in the tail (shown shaded) are localised in the sense of Anderson; that is to say they consist of traps with a continuous range of energies. A sharp value of the energy E_c separating localised from non-localised states, is predicted and is known as the "mobility edge". Electrons with energies below E_c move from one localised state to another by the process known as "thermally activated hopping"—each time an electron moves it has to receive energy from lattice vibrations and its mobility tends to zero with the temperature; an

electron above E_c can move as in a crystal without help from lattice vibrations or thermal energy, though with a mean free path often as small as the interatomic distance.

Evidence for the existence of a mobility edge comes from experiments on the drift mobility of electrons injected into various materials, particularly experiments by Spear⁷ and coworkers on silicon glow-deposited from silane. These, unlike most evaporated or sputtered films, do seem to fall on microscopic voids, the presence of which makes the application of the theory for a continuous medium open to doubt. But perhaps another class of phenomenon gives a clearer demonstration of the concept. These are phenomena in which in a solid or liquid there is a degenerate gas or free electrons, so that the material behaves in all respects like a metal, but in which, as in a liquid metal, the positions of the atoms lead to a random electric field.

In liquid metals the random field leads to random scattering of the electrons, and as in Ziman's theory² the scattering determines the mean free path. But—as is shown in Fig. 1, in the tail of a band the states are localised, and the electrical properties depend on whether the Fermi energy E_F , that is the energy of the highest occupied state, lies below E_c in the localised region, or above E_c . The conductivity of a metallic system depends only on the electrons with energies near the Fermi energy E_F , and so if E_F lies below E_c conduction is by hopping. I first showed that the conductivity as a function of temperature will then follow the law⁸

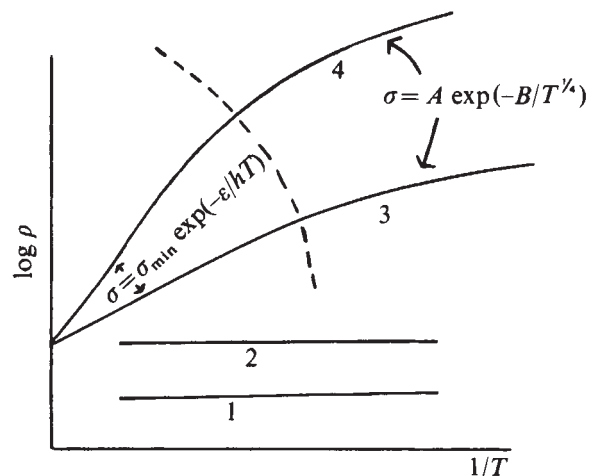
$$\sigma = A \exp(-B/T^{1/4}) \quad (1)$$

(where $T^{1/4}$ becomes $T^{1/3}$ in a two-dimensional system), a law which has been discussed extensively to find out in what conditions it should be valid. If on the other hand E_F lies above E_c , the conductivity tends to a finite value as the temperature tends to zero, which we can call metallic behaviour. If one then can vary the Fermi energy, for instance by varying the composition or the degree of disorder, or both, a kind of metal-insulator transition will occur, which has been named an "Anderson transition". The type of resistivity-temperature behaviour expected is shown in Fig. 2. When E_F lies below E_c , the conductivity σ at low temperature will follow the law (1); while at high temperatures current will be carried by electrons excited to E_c , so

$$\sigma = \sigma_{\min} \exp(-\varepsilon/kT), \quad \varepsilon = E_c - E_F$$

$\sigma_{\min}$, the lowest value of the conductivity for which there is no activation, is essentially that which one expects when the scattering is so strong that the mean free path becomes comparable

Fig. 2 Behaviour of the resistivity ρ of a substance in which there is a degenerate electron gas of which the Fermi energy can be shifted through the mobility edge. Disorder is increasing at E_F in the sequence numbered 1, 2, 3, 4.



with the distance between atoms (a). It is about $0.1e^2/\hbar a$, or in the neighbourhood of $1,000 \Omega^{-1} \text{ cm}^{-1}$. I have designated this quantity the "minimum metallic conductivity"⁸; the concept is not accepted by all theorists¹⁰ and there is a lively controversy at present on whether, as I believe, such a quantity exists.

There are many systems in which an Anderson transition can be observed, usually by using a series of specimens of varying composition or in heavily doped magnetic semiconductors by varying a magnetic field. But one of the neatest is the investigation due to Pepper^{11,12} and coworkers on conduction at the interface between n- or p-type silicon and a layer of silicon dioxide grown on the surface, using for instance the MOSFET (metal oxide silicon field effect transistor). In this device, by applying a voltage (the gate voltage) across the oxide layer, the number of electrons at the interface can be varied at will. Moreover these form a truly two-dimensional gas, and at helium temperatures the gas is degenerate. Also the oxide formed on the silicon contains positive and negative charges, distributed at random; therefore the electrostatic field seen by the electrons will vary in a random way, so that a "tail" is formed to the density of states, as in Fig. 1. By varying the gate voltage, therefore, and measuring the resistivity in the plane of the interface, it has proved possible to reproduce the behaviour of Fig. 2 in some detail. The experimental work, carried out in the Cavendish Laboratory in collaboration with Plessey Ltd, has two major achievements. Over four orders of magnitude the conductivity below 3 K has been found to be of the form

$$\sigma = A \exp(-B/T^n) \quad (2)$$

with $n = 0.32 \pm 0.02$, a measurement which established the two-dimensional nature of the phenomenon, which in all respects follows the predicted behaviour of Fig. 2; and secondly the minimum metallic conductivity can be established experimentally.

For a two-dimensional system the calculated value is about $0.1e^2/\hbar$, and it has been suggested by Thouless (unpublished) that this is a universal constant, not depending on the form of disorder; in conventional units this is about $10^{-5} \Omega^{-1}$.

The metal-insulator transition shown in Fig. 2 is essentially due to disorder and is quite different from the "Mott transition"^{13,14} which is first order and a consequence of interaction between electrons.

Conductivity having the forms (1) and (2) is known as variable-range hopping. It arises because the probability that an electron jumps from one localised state to another at a distance R is of the form

$$C \exp(-2\alpha R - (W/kT)) \quad (3)$$

where W is the energy difference between them and α is a constant. The greater R the greater the choice of state, so if the electron chooses the smallest available value of W ,

$$W \propto [1/(4\pi/3)] R^3 N(E_F)$$

Equation (1) comes from choosing R to maximise the quantity in equation (3). In two dimensions πR^2 replaces $(4\pi/3)R^3$, so equation (2) follows. Variable-range hopping has been extensively observed, but deviations from equation (1) occur in many cases, probably because the assumption made that C and $N(E)$ are sufficiently independent of energy and temperature may not be valid.

Experiments such as that described above give us a lot of confidence in the concept of a mobility edge in the conduction band of any disordered system, and in the possibility of applying it to real glassy materials, and indeed there is a multitude of papers in which this is done (see for instance ref. 13). One of the difficulties many theorists had in accepting Anderson's localisation idea, together with a continuous distribution of trap energies, was the intuitive feeling that an electron in a trap, if it tunnelled far enough, would find another trap with exactly the

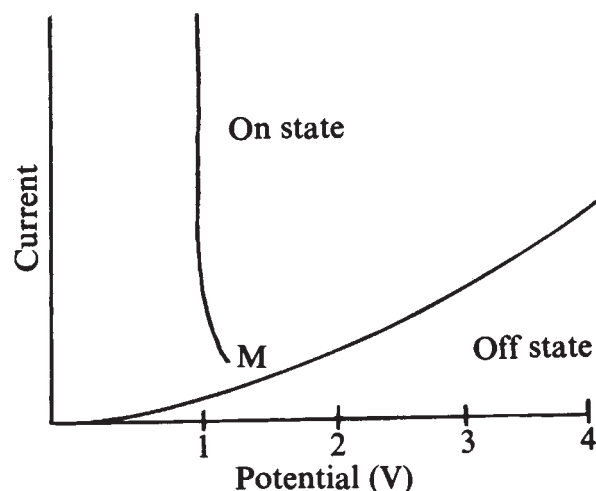


Fig. 3 Current-voltage curve for an ovonic threshold switch in on- and off-states.

same energy, and so the conductivity could not be temperature-activated and should not tend to zero at low temperatures. The reason why this is not so is rather subtle⁴; the experimental evidence from results such as those of Fig. 2 is, however, overwhelming. Also the measurements by Spear and coworkers⁷ of the drift mobility of injected electrons, that is to say the rate at which they drift across a specimen under the influence of an electric field, gives very firm evidence; at low temperatures the electron hops from one localised trap to another, at high temperatures it is excited to the mobility edge. But in many materials, particularly chalcogenides the drift mobility seems to depend on structural defects, and indeed we have little idea of their nature or whether it is even in principle possible to have a fully coordinated glass without them.

I feel that some of the most important developments in this field are likely to concern the kinds of structural defects in glasses, their effect on photoconduction and their relation to recombination centres in crystals. Even in crystals the mechanism ("multiphonon transitions") by which electrons and holes recombine without emission of radiation is imperfectly understood, in spite of much theoretical work; in silica glasses, we have very little idea. A challenging and controversial paper by Anderson¹⁴ may be relevant here, concerned with the way in which an electron in a trap deforms its surroundings. Also amorphous silica in silicon technology and amorphous tantalum oxide in anodic capacitors present a host of problems.

There is another problem which obstinately defies complete understanding, namely the mode of action of the threshold switch developed by S. R. Ovshinsky¹⁵ in his firm Energy Conversion Devices. In its simplest form this consists of a layer of STAG glass about $1 \mu\text{m}$ thick between two molybdenum electrodes. Figure 3 shows schematically how the switch behaves. In the off-state the material is a semiconductor with highly temperature-dependent impedance; in the on-state, which can be maintained as long as the current does not fall below M (the minimum holding current), the current is quite insensitive to temperature. It can reach more than 10 mA, probably in a channel a few μm thick. The controversy is about whether the on-state is due to a thermal instability, a hot channel at perhaps 500°C being formed¹⁶, or whether the channel is merely warm and electrons and holes are injected from the two electrodes¹⁷. A measurement of the temperature of the conduction channel would resolve the problem, but this has not proved possible; the radiation from a switch in the on-state has, however, been observed^{18,19} and bears no resemblance to black body radiation.

The controversy has been heated and at times bad-tempered, because the threshold switch was presented with commercial claims as something new, and some scientists in other industrial

laboratories claimed that it was a thermal instability and they had known about it for a long time. In spite of the many successes of the thermal theory I believe, though without absolute certainty, that there is indeed something new here, for which Ovshinsky and his many collaborators in American universities deserve credit. If electrons and holes are injected, degenerate gases of electrons and holes must be formed and the Fermi energies of one or other of them must lie above the corresponding mobility edge; otherwise the large observed currents with no thermal activation could not occur.

Indeed, just as this article is going to press, a good deal of new evidence is appearing in favour of an electronic and against a thermal model (ref. 20 and E. Peterson, G. W. Lake and D. Adler, unpublished).

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articles

How specific are nuclear “receptors” for thyroid hormones?

J. R. Tata

National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

The presence of “high-affinity-saturable” binding sites for thyroid hormones of similar characteristics not only in isolated nuclei but in all the major extranuclear cellular components, as well as the failure of cytosol to promote nuclear binding, invalidates the analogy with steroid hormone receptors and necessitates a more critical assessment of the physiological relevance of current approaches to binding of thyroid hormone in vitro to nuclear preparations.

THE identification of specific hormone-binding components or “receptors” in target cells is considered to be a primary requirement for an understanding of hormone action. It is now commonly held that polypeptide hormones exert their action by interacting with binding components on the surface of the cell¹, whereas some, but not all, small molecular weight hormones act through intracellular receptors². The latter generalisation is based largely on the concept of a two-step process of steroid hormone action whereby the hormone would first interact with a cytoplasmic receptor after which the hormone-receptor complex would be transferred into the nucleus where the complex or the hormone alone would initiate its physiological action²⁻⁶. Since steroid²⁻⁵⁻⁸ and thyroid hormones⁹⁻¹⁰, in common with other growth and developmental hormones¹¹⁻¹², markedly stimulate nuclear transcriptional activity, the concept of a nuclear site of specific hormone receptor(s) has recently been extended to thyroid hormones¹³⁻²³.

Early studies with cellular extracts revealed that both the “soluble” cytoplasmic fraction (“cytosol”) and various particulate subcellular fractions (mitochondria, microsomes, nuclei, debris, and so on) were equally effective in binding radioactive thyroid hormones *in vivo* or *in vitro*²⁴⁻²⁸. These

studies were performed with hormones of low specific radioactivity so that they could have only indicated nonspecific binding sites and not detected the easily saturable and physiologically more relevant binding sites or “receptors”. Recent studies on nuclear location of thyroid hormone receptors have been based on the use of ¹²⁵I-labelled tri-iodothyronine (T₃) of high specific radioactivity and these suggest that a rat liver nucleus has about 5×10^3 – 10×10^3 “specific” sites for this hormone^{15-17, 19-20}. Before these sites can be considered physiologically significant, however, it is essential to demonstrate that other subcellular components do not comprise sites of similar T₃-binding characteristics. In view of the importance of this question it may seem surprising that no studies have so far examined, in identical conditions, the thyroid hormone-binding characteristics of the major subcellular fractions derived from the same tissue, although the cytosol fraction is known to account for a large fraction of both high and low affinity sites in different tissues²⁴⁻³².

I shall describe here experiments which show that the binding of highly radioactive T₃ *in vitro*, as used in other recent studies, is not restricted to the nucleus or nuclear extracts, but that preparations of cytosol, plasma membrane, microsomes and inner mitochondrial membranes all exhibit similar “high-affinity” binding characteristics. The studies described below only deal with rat liver for the sake of comparison since most recent work on nuclear receptors is restricted to this tissue. All subcellular preparations exhibited two major classes of T₃-binding sites: (1) those with high affinity and relatively low capacity (commonly termed “specific”), and (2) those with low affinity and virtually non-saturable (“nonspecific”). This paper also describes a direct method for determining free and bound thyroid hormone on DEAE cellulose filter disks, adapted from a procedure described earlier for glucocorticoids³³.

Characteristics of binding

Adsorption of the ^{125}I - T_3 -protein complex on DEAE-cellulose gave a linear binding response for both particulate and soluble cellular components (Fig. 1). That the fraction of radioactivity retained on DEAE-cellulose filters represents T_3 bound to macromolecules was confirmed by precipitation and washing with trichloroacetic acid of identical samples. By treating the solution of radioactive T_3 with DEAE-cellulose just before use, the fraction of ^{125}I retained on "blanks" was reduced to below 4%. Any ^{125}I -iodide would thus be retained by the filters but not free T_3 or iodothyronine analogues. A major advantage of this procedure over those commonly in use is that the radioactivity measured on the adsorbent is a direct measure of the T_3 bound to protein or nucleic acids, whether the latter are in solution or not, and, therefore, more accurate than indirect methods involving the introduction of an

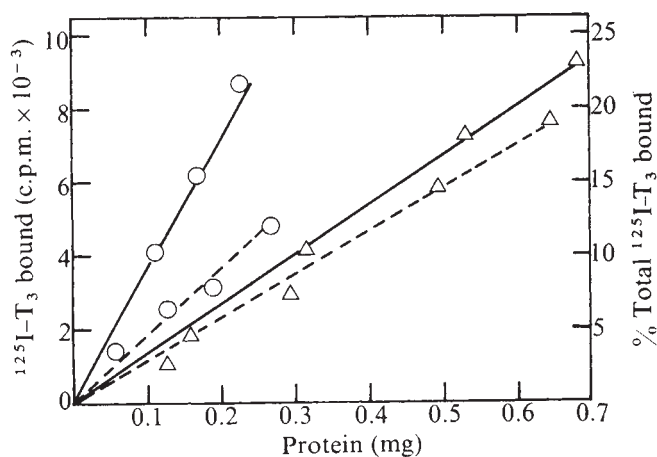


Fig. 1 Binding of ^{125}I -tri-iodo-L-thyronine (Abbott Laboratories, specific activity 322 Ci mmol^{-1}) to increasing amounts of nuclei and cytosol from livers of normal and thyroidectomised rats (150 g, Carworth Europe). Nuclei were freshly prepared from three livers by the method of Blobel and Potter³⁴, except that the homogenising medium was 0.25 M sucrose, 50 mM Tris-HCl ($\text{pH } 7.6$) 12.5 mM NaCl, 12.5 mM KCl and 5 mM MgCl_2 (STKNaM) and the nuclear pellet suspended in the same medium at a concentration of $4\text{--}6 \text{ mg DNA ml}^{-1}$. Cytosol was prepared from rat liver, perfused with 100 ml of Krebs-Ringer phosphate buffer, washed and homogenised in 2 vol of STKNaM. After two centrifugations at $10,000g$ for 20 min , the mitochondria-free supernatant was centrifuged at $105,000g$ for 3.5 h . The resulting supernatant, termed "cytosol", was stored at -30°C in small aliquots until use. Because of hormone adsorption problems, contact with glassware was avoided for all binding studies which were carried out in polycarbonate tubes, while all volumetric measurements were performed with polypropylene pipettes and syringes. Tissue preparations were incubated for 10 min at 37°C in a total volume of $260 \mu\text{l}$ made up with STKNaM. ^{125}I - T_3 was diluted with STKNaM to reduce the propylene glycol concentration down to below 5% . In this experiment, all samples were incubated with ^{125}I - T_3 at a final concentration of $2.5 \times 10^{-10} \text{ M}$ ($43,000 \text{ c.p.m.}$). In this and all other experiments, the diluted ^{125}I - T_3 solutions were passed through three layers of DEAE-cellulose filter disks (Whatman DE 81), presoaked in STKNaM, through a Millipore plastic Swinney adaptor attached to a 1-ml syringe just before incubation. This procedure removed ^{125}I -iodide and ensured low blanks. At the end of the incubation, the contents of the tube were immediately deposited on two layers of DEAE-cellulose disks, presoaked in 20 mM Tris ($\text{pH } 7.6$) and 1.5 mM EDTA held in a Millipore filter manifold. The vacuum was adjusted so that filtration took $10\text{--}20 \text{ s}$ only and the contents of the tubes and filters were rinsed $5\text{--}7$ times with 1 ml of Tris-EDTA solution. The filters, before they were dried, were crushed into polycarbonate tubes and ^{125}I determined in a Packard Auto-gamma counter. In all experiments, protein-free controls were processed simultaneously and corrections were made for blanks which were usually of the order of $2\text{--}4\%$ of the total input radioactivity. Protein, RNA and DNA were measured by standard procedures³⁵⁻³⁸. Each value is the mean of four determinations on two parallel incubations. The average variability was $\pm 6\%$. $\circ$, Nuclei; $\triangle$, cytosol; ---, normal; ———, thyroidectomised.

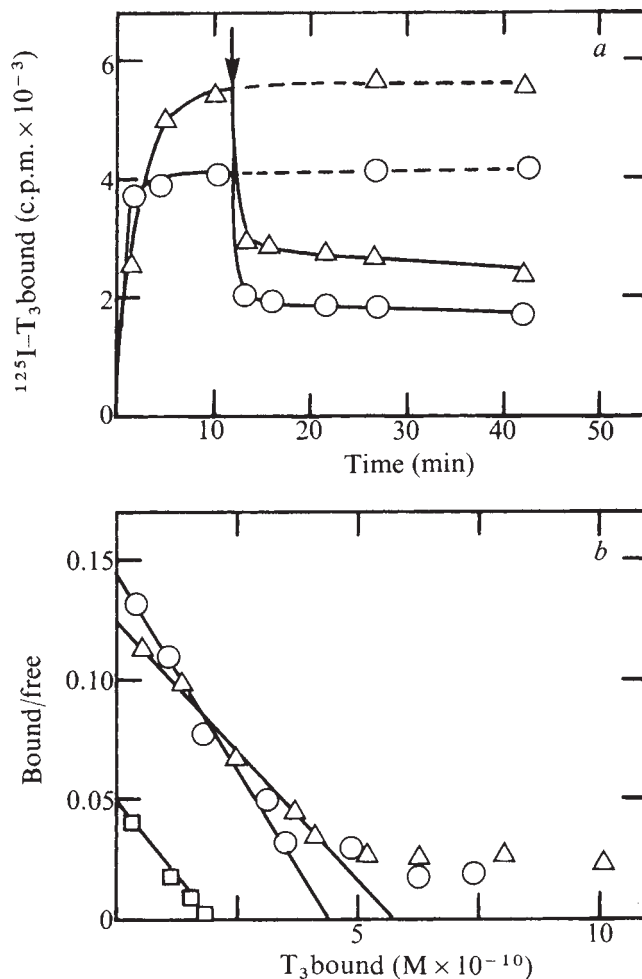


Fig. 2 T_3 -binding characteristics of nuclei, nuclear sap and cytosol from thyroidectomised rat liver. Nuclei and cytosol were prepared as described in Fig. 1 and nuclear sap as described in legend to Table 2. *a*, Time course and reversibility of binding. Cytosol (1.1 mg protein) and nuclei ($190 \mu\text{g protein}$) were incubated with 100 pg of ^{125}I - T_3 ($36,000 \text{ c.p.m.}$) at 37°C for different periods of time. To one set of tubes, $6 \mu\text{g}$ of non-radioactive T_3 were added at 12 min (indicated by the arrow) and the incubation continued for a further 30 min . To the other set no "cold" T_3 was added. The radioactive T_3 bound to cytosol or nuclei was determined as in Fig. 1. $\circ$, Nuclei; $\triangle$, cytosol. *b*, Scatchard plot analysis of affinity and capacity of binding of ^{125}I - T_3 to nuclei, cytosol, and nuclear sap. Different amounts of T_3 containing a fixed amount of ^{125}I - T_3 ($35,000 \text{ c.p.m.}$) were incubated with nuclei ($175 \mu\text{g protein}$), nuclear sap ($45 \mu\text{g protein}$) and cytosol ($236 \mu\text{g protein}$) for 10 min at 2°C . The other procedures are as described in Fig. 1. $\circ$, Nuclei; $\triangle$, cytosol; $\square$, nuclear sap.

insoluble "binder" of free hormone, such as dextran-coated charcoal.

All the physicochemical measurements in this work were made in parallel in normal and thyroidectomised animals since nearly half the liver nuclear T_3 -binding sites measured in thyroidectomised rats are occupied by endogenous thyroid hormone in normal animals^{17,20,38}. The binding of ^{125}I - T_3 at low concentrations ($2 \times 10^{-10}\text{--}5 \times 10^{-10} \text{ M}$) was very rapid ($T_{1/2}$ of $1\text{--}3 \text{ min}$) for both nuclei and cytosol alone (Fig. 2*a*). Displacement of radioactive hormone bound to these fractions by the addition of a large excess of unlabelled T_3 gave, at equilibrium, an approximate dissociation half time of 70 min for cytosol and 36 min for nuclei. ^{125}I - T_3 was also similarly displaced by higher concentrations of L-thyroxine and other iodothyronine analogues (not shown in Fig. 2*a*). When the binding of T_3 was measured at different hormone concentrations, it was found that for all cellular components from thyroidectomised liver, there exist at least two major classes of

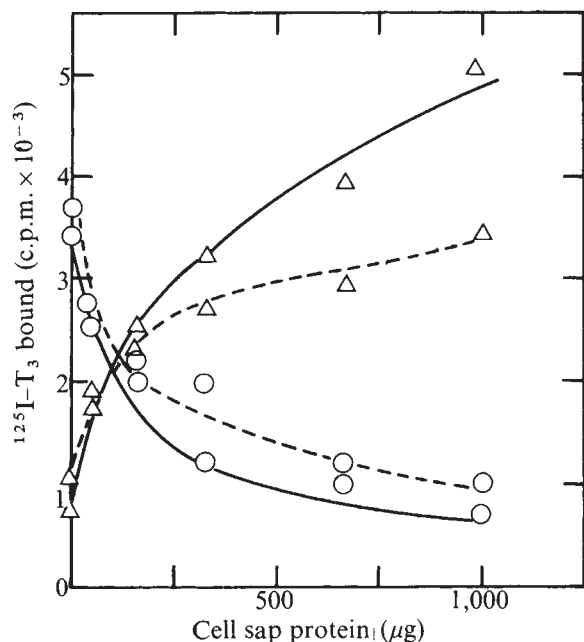


Fig. 3 Competition between cytosol and nuclei for the binding of $^{125}\text{I}-\text{T}_3$ at low and high concentrations of the hormone. A fixed amount of nuclei (180 μg protein) were incubated with different amounts of cytosol at 37°C for 10 min with either 4×10^{-10} M (17,000 c.p.m.) or 2×10^{-7} M (25,000 c.p.m.) $^{125}\text{I}-\text{T}_3$. At the end of the incubation, the nuclei were separated from the cytosol by centrifugation at 2,000g for 5 min and the bound radioactivity determined in each component by adsorption to DEAE-cellulose disks. Values for "O" cell sap are from incubations carried out in the absence of cell sap and therefore represent bound $^{125}\text{I}-\text{T}_3$ in the nuclear supernatant. Other conditions as in Fig. 1. O, Nuclei; Δ , cell sap; —, 4×10^{-10} M T_3 ; ---, 4×10^{-7} M T_3 .

binding sites: those that have high affinity and low capacity and those that exhibit a 100-fold lower binding affinity but are difficult to saturate. Data, such as those presented as Scatchard plots in Fig. 2b, gave values for association constant (K_a) of $1.2 \pm 0.3 \times 10^8 \text{ M}^{-1}$ and $0.8 \pm 0.3 \times 10^8 \text{ M}^{-1}$ for nuclei and cytosol respectively. The binding capacity for the same two fractions was $3.0 \pm 0.9 \text{ pmol}$ and $0.7 \pm 0.2 \text{ pmol}$ per mg protein, respectively. These values are within the same range as those reported elsewhere for nuclei^{15,17,18,20} and cytosol²⁸⁻³⁰ *in vitro*, especially when one takes into account the quite different methods used for studying binding.

Nucleus and cytosol

An outstanding characteristic of intracellular translocation of steroid hormones is the process by which the hormone has to be complexed to cytosol receptors before it can move into the nucleus. The receptor in the cytosol is different from the "acceptor" sites in the nucleus and the translocation of the hormone from one subcellular site to the other requires a temperature-sensitive activation step^{2-8,39}. In order to test the validity of a steroid hormone analogy for thyroid hormone

receptors, it was reasoned that if the same principle governed the two classes of hormones, then coincubating $^{125}\text{I}-\text{T}_3$ with nuclei in the presence of cytosol should enhance in a cooperative fashion the concentration of the hormone in the nucleus. On the other hand, an independent interaction between the hormone and the two subcellular fractions should lead to a competition between the subcellular components.

Figure 3 shows that the addition of increasing amounts of cytosol to nuclei led to a corresponding decrease in the radioactivity recovered with the nuclei. When one takes into account the binding constants and capacities given in Fig. 2b (see also Table 2) for the two fractions, the results can be accounted for by competition by the two subcellular fractions for the available T_3 , governed by simple mass action kinetics. Similar results were obtained at both low (4×10^{-10} M) and high (2×10^{-7} M) concentrations of the hormone, that is at concentrations above and below saturation of binding sites of high affinity and limited capacity. The results in Fig. 3 were obtained at 37°C so that a cytosol receptor activation step, analogous to that involving steroid hormones, should have given the opposite result. From Table 1 it is clear that preincubating cytosol at 37°C failed to enhance the uptake of the hormone into nuclei at either 2 or 37°C . Many permutations, to be reported elsewhere, of coincubating nuclei and cytosol at different temperatures also failed to reveal a temperature-dependent activation step for a cytosol-nuclear transfer mechanism. Although they did not specifically look for a thermosensitive activation step, Surks *et al.*¹⁷ and De Groot and Torresani¹⁸ also concluded that the cytosol did not play a role in the transfer of the hormone to the nucleus. The steroid hormone analogy for early events in hormone action is clearly not applicable to thyroid hormones.

Other cellular components

The above findings of not too dissimilar classes of competing binding sites in the nucleus and soluble cytoplasm raised the question of their specificity with respect to other cellular components. An examination of all the major subcellular and subnuclear fractions was undertaken in which the physicochemical characteristics for $^{125}\text{I}-\text{T}_3$ binding were measured simultaneously and under identical conditions. Very rigorous conditions were established in the preparation of subcellular fractions to eliminate mutual contamination. Thus, a subfraction of plasma membranes comprising only the outer face of the cell surface⁴¹ was used in order to exclude microsomal contamination. Since the outer membrane of mitochondria shares some common components with the endoplasmic reticulum⁴², a preparation of only the inner mitochondrial membrane⁴³ was used. Similarly, nuclei were washed with an amount of detergent (0.2% Triton) known to remove both the outer and inner nuclear membranes⁴⁴, again in order to eliminate microsomal contamination. Recently, many reports have indicated that the nuclear site for thyroid hormone binding is in the chromatin from which it can be extracted with 0.4 M KCl^{14,17,20-22}. Such treatment of chromatin is known to extract the bulk of non-histone acidic nuclear proteins⁴⁵ as well as acceptor sites for oestrogen, progesterone, androgen and corticosteroids in their respective target nuclei^{2,4}.

Table 1 Absence of temperature-dependent activation step for transfer of bound $^{125}\text{I}-\text{T}_3$ from cytosol to nuclei

Temperature of preincubation of cytosol + $^{125}\text{I}-\text{T}_3$ ($^\circ\text{C}$)	Temperature of incubation of cytosol + nuclei ($^\circ\text{C}$)	Bound $^{125}\text{I}-\text{T}_3$ (c.p.m.) recovered in	
		Nuclei	Cytosol
2	2	1,923	2,422
2	37	1,793	2,479
37	2	2,026	1,941
37	37	1,701	2,294

Cytosol from thyroidectomised rat liver (1 mg protein in 0.16 ml of STKNaM) was preincubated with 2×10^{-10} M $^{125}\text{I}-\text{T}_3$ (35,000 c.p.m.) for 10 min at either 2 or 37°C and then chilled in ice for 5 min. 0.1 ml of a freshly prepared nuclear suspension (275 μg protein) was then added and the mixture incubated for 10 min at 2 or 37°C after which nuclei and cytosol were separated as indicated in Fig. 3. The fraction of $^{125}\text{I}-\text{T}_3$ bound was determined as described in Fig. 1.

Table 2 Characteristics of binding of ^{125}I -tri-iodothyronine to nuclear and extranuclear components

Component	Rat	Association constant K_a (M^{-1})	No. of binding sites M (pmol per mg protein)	$T_{1/2}$ (min) for Association	$T_{1/2}$ (min) for Dissociation
Nuclei	Normal	2.4×10^8	1.73		
	Thyrex	1.3×10^8	2.96	2.9	36
Cytosol	Normal	8.0×10^7	0.67		
	Thyrex	2.3×10^8	0.69	3.0	70
Nuclear sap	Thyrex	1.7×10^8	2.10	5.9	
	Thyrex	9.3×10^7 *	0.15	8.5	
0.4 M KCl Extract of chromatin	Normal	7.3×10^7	0.27	7.5	20
Inner mitochondrial membranes	Normal	8.2×10^8	0.32		
Plasma membranes	Thyrex	5.9×10^8	0.08	8.0	80
Microsomes					

Nuclei and cytosol were prepared as described in Fig. 1. Nuclear sap was prepared by gentle sonication of nuclei according to the method of Tata and Baker⁴⁶. The chromatin pellet remaining after removal of nuclear sap was extracted twice at 2 °C with 0.4 M KCl (chromatin from 3 g equivalent liver ml^{-1}) and both the 0.4 M KCl extract and nuclear sap were dialysed overnight against three changes of 200 vol of STKNaM. Much of the protein in 0.4 M KCl extract precipitated out during dialysis and it was redissolved in 0.4 M KCl. Both the nuclear sap and the salt extract of chromatin were concentrated in an Amicon (Minicon B15) dialysis concentrator to a protein concentration of 1–2 mg ml^{-1} . Plasma membranes were subfractionated to yield the outer face components according to Wisher and Evans⁴¹ and the inner mitochondrial membranes prepared by the method of Greenawalt⁴². Microsomes were the pellet obtained from the preparation used for obtaining cytosol. The binding characteristics were calculated according to the details given under Figs 1 and 2, except that the standard temperature was 37 °C. Nuclei, when not fractionated, were washed once with 0.2% Triton X-100 followed by two washes with STKNaM. Thyrex: thyroidectomised.

*The values for 0.4 M KCl extract of chromatin are rough estimates only because of the problem of protein precipitation in STKNaM.

Similar preparations were, therefore, also made from rat liver chromatin but after previous removal of "nuclear sap" which contains soluble nuclear proteins released by gently disrupting nuclei at isotonic salt concentration⁴⁶. Since some subcellular components of liver degrade thyroid hormone²⁸, comparison of thyroid hormone binding to different subcellular fractions was carried out both at 37 and 2 °C.

The results, summarised in Table 2, show that all subcellular fractions exhibited the presence of apparently "specific" and readily saturable T_3 -binding sites. Their relative affinities varied only over a range of ± 5 -fold, which is not considerable when considering that one is comparing binding of a ligand to macromolecules in solution for some components and in a suspension of membranes or complex macromolecular particles in others. The number of T_3 -binding sites per nucleus was of the order of 10,000 but the total binding capacity as expressed per mg protein or per g equivalent of tissue varied over a wide range for the different fractions. As regards fractionation of nuclei, in results to be presented elsewhere (J.R.T., K.M.B.S., and B.B., unpublished observations), more than half the ^{125}I - T_3 bound to nuclei was recovered in the euchromatin fraction. This is in agreement with recent reports from Baxter's laboratory^{23,40} but whether or not the endogenous hormone is directly bound to DNA as concluded by these workers or to non-histone protein in intact chromatin, as proposed by others^{14,15,20–22}, is difficult to decide. In our hands much difficulty was experienced in studying the binding under standard ionic conditions to the non-histone proteins extracted from chromatin by 0.4 M KCl because of precipitation of protein on lowering the salt concentration. On the other hand, the nuclear sap fraction, which represents soluble nuclear proteins released on gentle sonication of nuclei at relatively low salt concentrations⁴⁶, had T_3 -binding sites present at higher concentration (as expressed per mg protein) than in total euchromatin (J.R.T., *et al.*, unpublished). These findings (Table 2) of the ubiquitous distribution of T_3 -binding sites in nuclear and extranuclear fractions of rat liver are compared in Table 3 with the characteristics described by other workers for this and other tissues, thus establishing that such sites are certainly not restricted to the nucleus.

Hormone binding and action

The above results raise several questions concerning the relevance of binding of thyroid hormone to subcellular components *in vitro* to the initiation of the physiological actions of the hormone. First, do the T_3 -binding component(s) of K_a of 10^8 – 10^9 M^{-1} in the different subcellular fractions represent the same or different molecular species? An electrophoretic

or sedimentation analysis of the solubilised binding components of cytosol and nuclear sap would provide a partial clue. Whatever the answer, it seems that the emphasis given in recent work to nuclear binding of T_3 *in vitro* to explain thyroid hormone action is unjustified nor is the steroid hormone analogy valid. Second, the quantitative aspects of the binding raise some doubts about the relevance to physiological action. Except for the pituitary³⁸, only about 15–20% of the endogenous or exogenous hormone is associated with the nuclei from liver or kidney homogenates^{16,21,38}. The "specific" high-affinity binding of T_3 to various subcellular fractions reported here and elsewhere^{13–23,27–32,38}, is not much different from that described in our earlier studies^{27,28} with hormone of low specific radioactivity binding mostly to the low-affinity, non-saturable sites. Moreover, both the high-affinity and low-affinity sites in the nucleus and elsewhere do not exhibit a stringent specificity with respect to the biological activity of thyroid hormone analogues. In fact, the relative affinities of various analogues reported for the easily saturable, high-affinity sites^{18,21,30,31} are very similar to those reported some years ago for the low-affinity, "nonspecific" sites^{27,28}. On the other hand, a high degree of correlation between physiological potency or anti-hormone activity has been a major argument in supporting the physiological relevance of nuclear binding of steroid hormones^{2,4,6–8,47,48}. The figure of 5–10,000 binding sites for T_3 per rat liver nucleus may also be too high if one considers the phenomenon in terms of specific induction of proteins through genetic activation. The affinity of the nucleus for tri-iodothyronine is not much greater than that exhibited by cytoplasmic fractions or of plasma thyroxine-binding globulin (TBG) for either tri-iodothyronine or thyroxine ($K_a = 2.0 \times 10^9$ M^{-1} and 2.4×10^{10} M^{-1} , respectively)²⁸. Oppenheimer *et al.*¹⁶ calculated the affinity of the rat liver nucleus for T_3 *in vivo* as K_a of 4.7×10^{11} M^{-1} , a value nearly 1,000 times higher than that reported from the same laboratory¹⁷ for the "specific" interaction *in vitro*. Interestingly, intact lymphocytes bind T_3 with a K_a of 9.4×10^{11} M^{-1} (binding capacity = 6.5×10^{-4} pmol per 10^7 cells) but the location of the binding sites has not been described⁵⁰. The discrepancy between the relative affinities of nuclei *in vivo* and *in vitro* has been explained on the basis of the deleterious effects of ionic and other conditions *in vitro*¹⁷ but then the same argument should also apply to extranuclear fractions. There is, in fact, no direct evidence that the binding to nuclei *in vitro* tells us much about the properties of a possible true nuclear receptor for thyroid hormone that may be present in much lower concentration. Similar questions have been raised for extremely high-affinity nuclear binding for oestradiol present in amounts as low as

Table 3 Characteristics of binding of ^{125}I -tri-iodothyronine to "specific" high affinity sites in nuclear and cytosol preparations described by other workers

Preparation	K_d (M^{-1})	No. of sites	$T_{1/2}$ (min) for		Ref.
			Association	Dissociation	
Rat liver nuclei	6.1×10^8	2.0 pmol g^{-1} liver	3	14	15
	5.5×10^8	1.3 pmol g^{-1} liver			17
	2.0×10^9	0.8 pmol g^{-1} liver			18
Rat liver NHP*	1.6×10^9	6,000 per nucleus			22
Rat pituitary tumour cell line nuclei	2.9×10^8	5,000 per nucleus			19
Rat liver cytosol	4.3×10^7	0.53 pmol mg^{-1} protein			30
Rat kidney cytosol	2.5×10^7	2.9 pmol mg^{-1} protein			30
Dog liver cytosol	2.3×10^7	—	4	50	29
Pig pituitary cytosol	4.0×10^8	—			28

*NHP, Non-histone protein (nuclear proteins soluble in 0.4 M NaCl).

10–1,000 molecules per cell in the uterus^{8,51,52}. It is hard to visualise how physiologically important receptor molecules could be detected at that level for thyroid hormones by binding techniques currently available.

High-affinity steroid-binding sites have also been recently detected in extranuclear sites (plasma membranes, microsomes) in tissues where similar nuclear binding sites have been well characterised^{53–55}. An ubiquitous distribution of not only thyroid but also steroid hormone-binding sites leads to a consideration of possible multiple sites of initiation of actions of growth and developmental hormones. It is well known that multiple^{9–11,56} or "pleiotypic"⁵⁷ responses are elicited by all growth and developmental stimuli, whether they be hormonal or not, before induction of new protein or messenger RNA species. Many of these effects, such as the alteration of cell surface, permeability towards small molecules and ions, metabolism of cyclic nucleotides, and so on cannot be accounted for by an action exclusively initiated at the level of the cell nucleus. The generalisation that polypeptide hormones act at the cell surface whereas small molecular weight hormones act within the cell is a gross over-simplification, as far as the latter class of hormones are concerned; after all, most actions of catecholamines can be satisfactorily explained on the basis of their interaction with plasma membrane receptors associated with adenylate cyclase⁵⁸. One has, therefore, to consider the possibility that the final expression of a growth and developmental hormone may result from a cooperative action generated by simultaneous or sequential but independent interactions with more than one nuclear and extranuclear sites.

The above discussion does not diminish the role of the nucleus in the action of thyroid or steroid hormones nor of the importance of continuing with hormone-binding studies. Nor is it implied that receptors or acceptors of steroid hormones are of little consequence in hormone action. Indeed, increasing evidence is now gathering from genetic studies that the cytosol–nuclear translocation and the nuclear localisation of steroid hormone are intimately associated with their capacity to induce the synthesis of specific proteins^{59,60} and a similar analysis of responsiveness to thyroid hormones would help clarify some of the ambiguities raised in this paper concerning their binding to nuclear and extranuclear cellular components. What can be said is that thyroid hormones do not interact with their target cells in the same manner as do steroids and that the current emphasis on their nuclear site of action based on binding studies with tri-iodothyronine *in vitro* may not reveal a balanced picture of the location and distribution of true receptors and, therefore, the initial events leading to the physiological action of these hormones.

Detailed studies on the subcellular and subnuclear distribution of thyroid hormone binding sites will soon be published elsewhere.

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A major geothermal anomaly in the Gulf of California

Lawrence A. Lawver

University of California, San Diego, Marine Physical Laboratory of the Scripps Institution of Oceanography, La Jolla, California 92037

David L. Williams

US Geological Survey, Denver, Colorado 80225

Richard P. Von Herzen

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

We have mapped a 3-km wide, high heat flow anomaly with a maximum value of $30 \mu\text{calorie cm}^{-2} \text{ s}^{-1}$ within a zone of seafloor extension in the central Gulf of California. From seismic reflection data and thermal modelling we suggest that the anomaly is caused by a 1-km wide basaltic intrusion which is roughly 100 m deep and less than 18,000 yr old.

SEAFLOOR spreading associated with the East Pacific Rise can be extended to inside the mouth of the Gulf of California, by correlating magnetic lineations¹. Further into the gulf the magnetic lineations are either disturbed by the thick sedimentary cover, or the lineations are weak or non-existent due to an intrusive or cooling mechanism that is unfavourable to their formation². The San Andreas fault to the north is recognised as the major part of the boundary between two large plates—the Pacific and North American. The Gulf of California is a transitional region between predominantly seafloor spreading to the south and totally transform fault motion to the north. The lack of identifiable magnetic anomalies in the gulf makes it necessary to look for other evidence to support the idea of seafloor extension. The pole of rotation for the Pacific–North American plates is 50.9°N , 66.3°W (ref. 3). The trend of the gulf differs sufficiently from a small circle about this pole to require *en echelon* offset of the transform faults and thus requires some seafloor extension in the gulf. Large heat flow⁴ and microseismicity⁵ in the basins also suggest that the gulf is undergoing extension as well as transform fault motion.

Shepard⁶ recognised the similarity between the Gulf of California and the Red Sea and mapped the *en echelon* strike-slip faults and rhombic nature of the basins. Rusnak, Fisher and Shepard⁷ related the opening in the gulf to the strike-slip motion of the San Andreas fault and deduced a figure of 260 km of opening, with Cabo San Lucas originally near Banderas Bay, Sinaloa. They considered the central depressions in the Guaymas and Farallon Basins to be extensional features due to their perpendicularity to the strike-slip faults. Larson¹ deduced a half-spreading rate of 30 mm yr^{-1} by correlating magnetic anomaly patterns at the mouth of the gulf. Lawver *et al.*⁴ reported on 13 new heat-flow measurements in the Guaymas Basin bringing the then total to 16, with three from Von Herzen⁸. Large values were found in the north-east central Guaymas Deep, $>5 \text{ HFU}$ ($>200 \text{ mW m}^{-2}$) with the greatest being 7.2 HFU (300 mW m^{-2}) near a 160-m high mound in the deep.

Measurements and techniques

The most recent cruise, in October 1974, collected 58 new heat-flow measurements in the Guaymas Basin. Most of the work

was concentrated in the north-east Guaymas Deep but some of the most interesting results came from the south-west Guaymas Deep (Fig. 1).

The thermal gradients were measured using the Woods Hole Oceanographic Institution multipenetration heat-flow probe⁹. It consists of a 2.5-m probe with three outrigger thermistor probes at 1-m intervals. Acoustically telemetered data were recorded on a precision depth recorder aboard the RV Agassiz of the Scripps Institution of Oceanography. Navigation consisted of radar fixes and depths later plotted on the satellite navigated bathymetric chart of G. F. Sharman (unpublished). The radar navigation was accurate to about 500 m, with relative positions between individual measurements being better ($\sim 250 \text{ m}$), see Fig. 2. The sediments in the Guaymas Basin are extremely uniform and consist almost solely of green hemipelagic mud. Thermal conductivities were assumed from measurements made on previous cruises⁴. The water content is very high in the near-surface sediments causing the conductivity in those shallower than 50 cm to be about $1.5 \times 10^{-3} \text{ calorie } ^\circ\text{C}^{-1} \text{ s}^{-1} \text{ cm}^{-1}$ ($0.60 \text{ W m}^{-1} \text{ K}^{-1}$). The conductivity below 50 cm is assumed to be reasonably constant, based on the findings of Lawver *et al.*⁴ and is taken to be $1.71 \times 10^{-3} \text{ calorie } ^\circ\text{C}^{-1} \text{ s}^{-1} \text{ cm}^{-1}$ ($0.71 \text{ W m}^{-1} \text{ K}^{-1}$). The heat-flow values are shown on the diagram in Fig. 3 and listed in Table 1.

The average sedimentation rate in the central part of the Guaymas Basin is approximately 2.0 m per 1,000 yr (ref. 10). This high sedimentation rate depresses the surface heat flow, requiring approximately a +12% correction according to Fig. 9 of ref. 11. From the seismic reflection profiles in Fig. 5, the south-west Guaymas Deep seems to be underlain by basement which has intruded through older sediments, as evidenced by the apparent $0.8 \pm 0.2 \text{ s}$ thick sediment to the north and $0.6 \pm 0.1 \text{ s}$ thick sediment to the south. Later we show that the heat-flow anomaly is consistent with an intrusion of this geometry with an age of about $2 \times 10^4 \text{ yr}$. For this model, the anomaly is completely dominated by the transient cooling of the intrusion and the sedimentation rate has a negligible influence. For purposes of discussion no corrections will be made to the recorded heat-flow values.

Interpretation

Nearly every other region of active seafloor generation is almost devoid of sediments making heat-flow measurements there possible only in isolated sediment ponds. The available heat-flow values from these regions are apparently depressed by hydrothermal circulation. The heat-flow values measured in the Gulf of California are significantly higher than those measured on other spreading centres. Henyey (unpublished) measured a

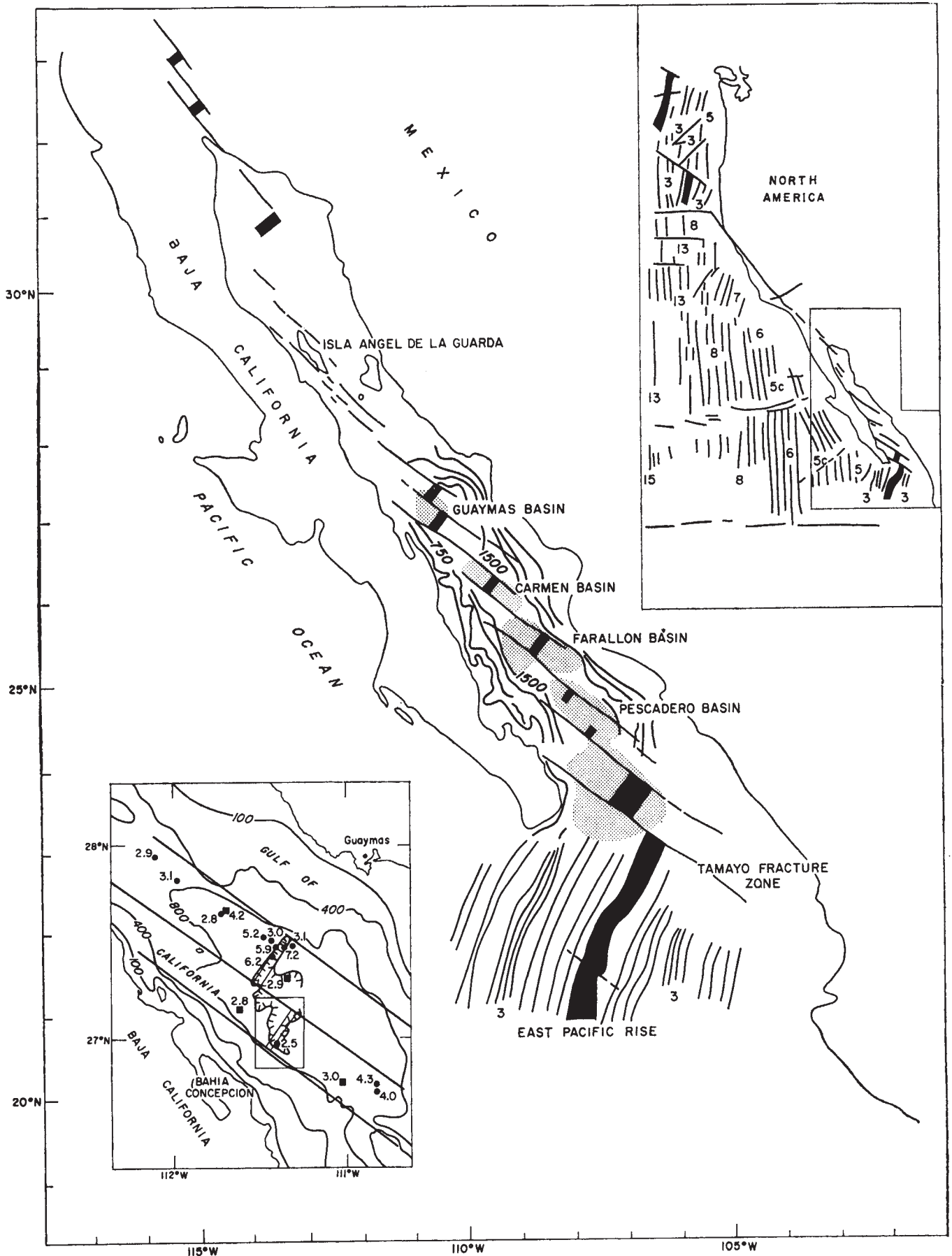


Fig. 1 Lineated magnetic anomalies at the mouth of the Gulf of California, from Larson¹. Generalised bathymetric lines in gulf in metres. Upper inset map with magnetic anomaly numbers from Atwater²⁰. The lower inset is a generalised diagram of the Guaymas Basin from Lawver *et al.*⁴ showing heat-flow values, in $\mu\text{calorie cm}^{-2} \text{ s}^{-1}$. Squares indicate values from Von Herzen⁸. Circles from Lawver *et al.*⁴. Box shows area of detailed heat-flow measurements shown in Fig. 2. Heavy lines and stippled areas mark transform faults and areas of active generalised extension, respectively.

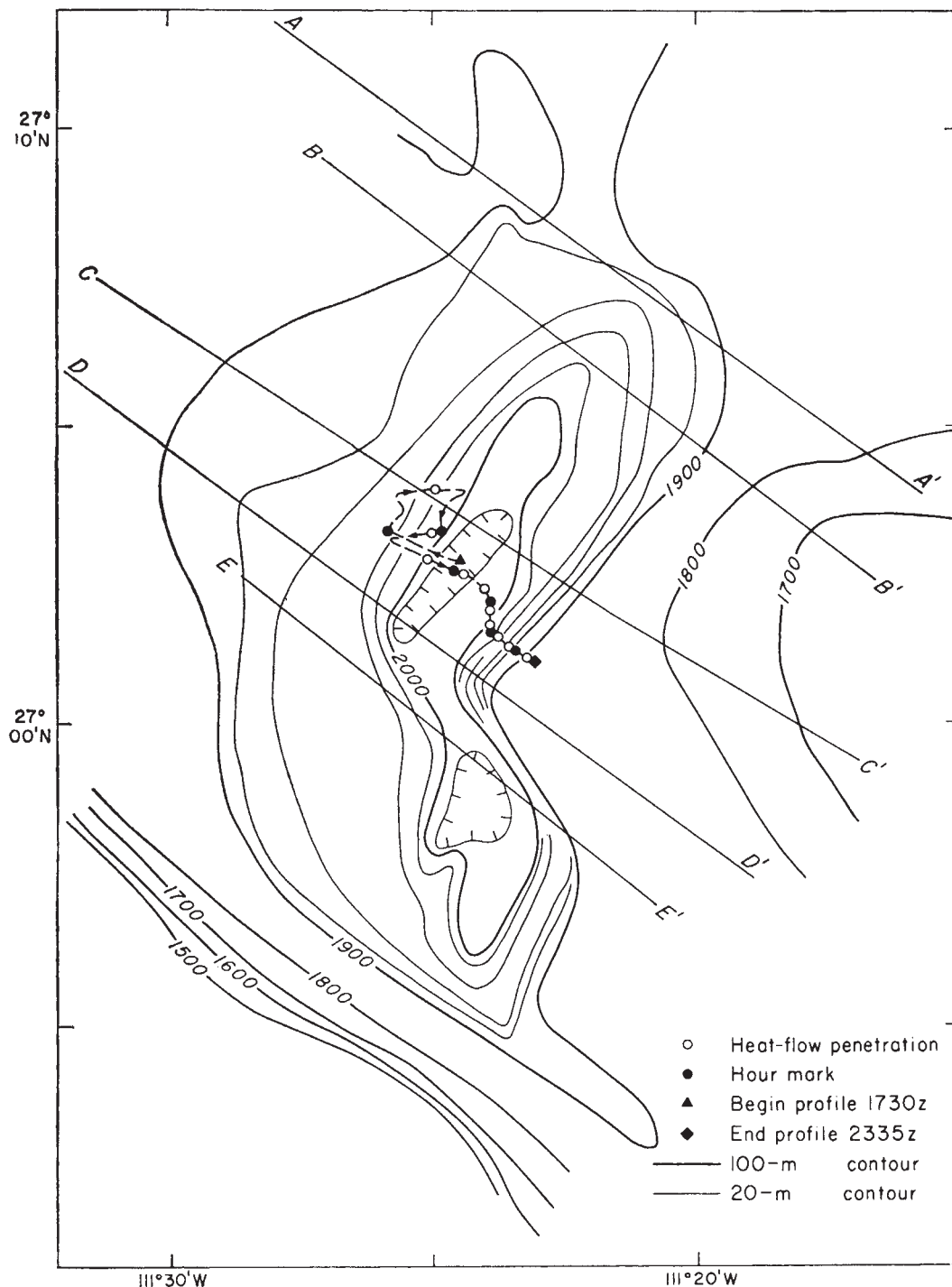


Fig. 2 Detailed chart of heat-flow survey of the south-west Guaymas Deep in corrected metres. Bathymetry from G. Sharman (unpublished). The values progressed from the north-west side of the depression to the south-east side. Seismic reflection profiles A-A' to E-E' indicated by solid lines are shown in Fig. 5.

value of 35.8 HFU ($1,450 \text{ mW m}^{-2}$) in the Ballenas Channel in the northern gulf but this may be an isolated hot spring.

The heat release associated with lithospheric creation for a spreading rate equivalent to that of the gulf is estimated to be at least 330 calorie s^{-1} per cm of ridge length within 35 km of the spreading centre¹² or an average heat flow of 47 HFU. At most oceanic spreading centres the rate is presumed much higher near the centre due to the faster cooling produced by hydrothermal circulation (compare ref. 12). The Gulf of California is different because the actively spreading zone may be losing heat through the sea floor to a greater extent by conduction because of its unusually thick and continuous sedimentary cover.

Figure 4 shows all the 26 published and new heat-flow values in the Guaymas Basin plotted against distance from the presumed most recent spreading centre. Additional heat-flow

values in the Guaymas Basin to be discussed in a subsequent paper reinforce the basic shape of the curve in Fig. 4. It is unusual that the background heat flow for the Guaymas Basin is remarkably regular at 3.4 ± 0.5 HFU for the total length of the basin. The central deep coincides with a higher heat-flow zone less than 20 km wide.

This distribution of conductive heat flow does not fit the theoretical models for a conductively cooled lithosphere (see ref. 13). As can be seen in Fig. 4, the observed heat-flow values average less than half of the predicted value. Two factors, prevalent in the Gulf of California, would tend to make the measured values lie below the theoretical curve. First, the theoretical models assume continuous intrusion whereas in the Gulf of California there is evidence that the location of spreading centres changes discontinuously with time. This idea was first advanced by Bishoff and Henyey¹⁴ on

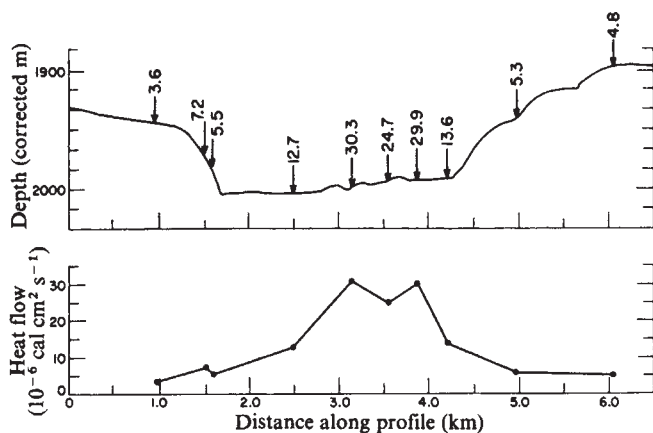


Fig. 3 Profile across the Guaymas Deep showing the heat-flow (HFU) values and the shape of the anomaly found.

the basis of seismic reflection profiles. It is supported by our heat-flow measurements and the reflection profiles shown in Fig. 5. At (a) on profile D-D' (Fig. 5), there is a slight depression which has not been completely filled with sediment; it has an uncertain age relationship to the central intrusion. Probably the depressions are in fact grabens caused by the pulling apart of the basement, resulting in an intrusion and a thinning of the overlying sediment (Fig. 3, ref. 21). The weight and shear strength of the sediments compared with water may cause the intrusion to slow and stop before it reaches the surface. This effect of the sediment may even cause the site of intrusion to change discontinuously rather than remaining fixed as at a normal spreading centre. The second factor that may affect the heat flow is that the ratio of the widths of spreading centres to the length of transform faults is much less in the Gulf of California than in more normal oceanic spreading areas. This could lead to significant lateral heat transfer. Although these factors redistribute heat to the flanking regions and away from the actively spreading locations, the total heat loss remains approximately the same and thus will not explain the great discrepancy between the predicted and observed heat flows.

Assuming that seafloor spreading applies in the gulf, the most plausible explanation for the discrepancy is hydrothermal heat loss even though there is a thick sediment cover. The existence and importance of hydrothermal convection in the basement have been discussed by numerous investigators (see reference lists in refs 12 and 15). The porosity and permeability would be related to dike contacts, the dissolution of minerals by thermal waters, the horizontal component of thermal contraction, and faulting. In the gulf the evidence is not as compelling as it is at other spreading centres. There is no evidence of numerous low heat-flow values and the large scatter in ob-

served values that have supported previous hydrothermal circulation hypotheses. We made approximately 50 km of near-bottom horizontal water temperature profiles (see ref. 12) in the north-east Guaymas Basin but failed to detect any temperature anomalies that might be associated with hydrothermal vents. Evidence for sediment that was more metalliferous than normal was found only in the southernmost basin of the gulf (P. Wilde, personal communication). Since this basin is nearest to the East Pacific Rise and has a much thinner sediment cover¹⁶ it is more likely to be subject to hydrothermal discharge through the sediment surface. In the heavily sedimented Guaymas Basin, where the sediments are assumed to be relatively impermeable and there is very little expression of faulting in the surface sediments, it is hard to imagine the free convection that Williams *et al.*¹² found on the Galapagos spreading centre.

Thermal modelling

With the line drawings of seismic reflection profiles crossing the south-west Guaymas Deep (shown in Fig. 5), available as control, the heat-flow data were inverted under the assumption of conductive cooling. We first used Horai's model¹⁷ for a dike intruded instantaneously and remaining at a constant temperature for infinite time. This model yields a 1.6-km wide intrusion with a depth to its top of 220 ± 30 m, using a background heat flow of 3.4 ± 0.5 HFU. But this gives either an unacceptably high surface thermal gradient (5°C cm^{-1}) or a temperature too low for molten rock (400°C). This result implies that the measured heat-flow values are not due to a constant temperature intrusion but rather to a transient cooling process. Therefore, we used Simmons¹⁸ method for modelling the transient cooling of a dike, which is a variation of that given by Carslaw and Jaeger (ref. 19, p. 256). The temperature for a dike is given as:

$$T(x, y, z, t) = (S/8) E(x, x_1, x_2) E(y, y_1, y_2) \{ E(z, z_1, z_2) + E(z, -z_1, -z_2) \}$$

where S = source strength, which for a three-dimensional source is simply the temperature at the time of the intrusion, here assumed to be $1,100^\circ \text{C}$; x_1 and x_2 are coordinates defining the width of the dike; y_1 and y_2 give the lateral extent of the dike; z_1 and z_2 are the depths to the top and bottom respectively and E is a dimensionless function defined as:

$$E(\xi, \xi_1, \xi_2) = \text{erf} \frac{\xi - \xi_1}{(4kt)^{1/2}} - \text{erf} \frac{\xi - \xi_2}{(4kt)^{1/2}}$$

where t is the time after intrusion and k is thermal diffusivity. If one assumes y_1 and y_2 are large, greater than $3(4kt)^{1/2}$, then $E(y, y_1, y_2)$ is two. For a time of 10^4 yr the absolute value of y_1 or y_2 would have to be larger than 0.8 km. Using Figs 2 and 5, it seems that $y_1 = -2.0$ km and $y_2 = 6.0$ km. z_2 is the

Table 1 Heat-flow data

Station no.	Latitude (°N)	Longitude (°W)	Water depth (corrected m)	T	P	N	K	Q
12.1	27°04.0'	111°24.4'	2,004	2.82	2.7	3	1.71 (0.72)	5.5 (230)
12.2	27°03.6'	111°24.9'	1,988	2.82	2.7	3	1.71 (0.72)	7.2 (301)
12.3	27°03.1'	111°25.8'	1,955	2.82	2.7	3	1.71 (0.72)	3.6 (151)
12.4	27°02.6'	111°24.6'	2,024	2.83	2.7	2	1.71 (0.72)	12.7 (531)
12.5	27°02.4'	111°24.3'	2,020	2.82	2.7	1	1.71 (0.72)	30.3 (1268)*
12.6	27°02.2'	111°24.0'	2,014	2.82	2.7	1	1.71 (0.72)	24.7 (1033)*
12.7	27°02.0'	111°24.0'	2,012	2.82	2.7	1	1.71 (0.72)	29.9 (1251)*
12.8	27°01.8'	111°23.9'	2,010	2.82	2.7	3	1.71 (0.72)	13.6 (569)
12.9	27°01.5'	111°23.7'	1,952	2.83	2.7	3	1.71 (0.72)	5.3 (222)
12.10	27°01.3'	111°23.4'	1,898	2.83	2.7	3	1.71 (0.72)	4.8 (201)

T is bottom water temperature ($^\circ \text{C}$); P is estimated sediment penetration (m) of the lowermost probe used in gradient measurements; N is the number of thermistors used for sediment temperature gradient measurements; K is the thermal conductivity in 10^{-3} calorie $^\circ \text{C}^{-1} \text{cm}^{-1} \text{s}^{-1}$ ($\text{W m}^{-1} \text{K}^{-1}$) (All values are assumed); Q is the heat flow in 10^{-6} calorie $\text{cm}^{-2} \text{s}^{-1}$ (10^{-3}W m^{-2}).

*Only one thermistor on scale.

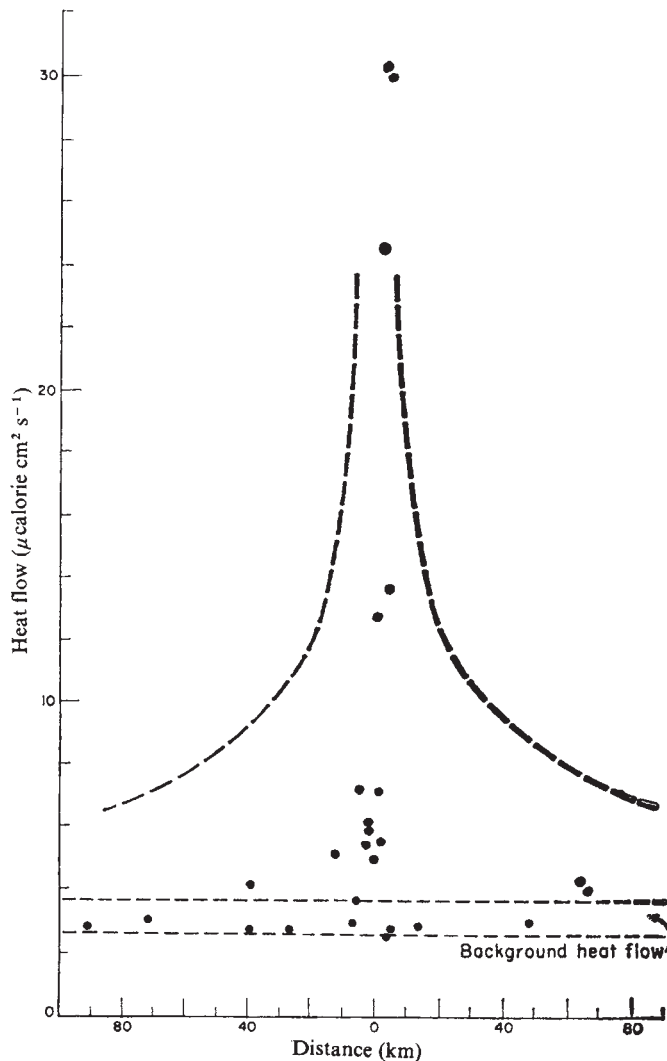


Fig. 4 Summary of the previous published values and the ten new values. The background heat flow is roughly 3.4 ± 0.5 HFU (135 ± 20 mW m $^{-2}$). The heat flow anomaly is strikingly regular directly over the depressions confirming them as the locus of present intrusive activity.

depth to the base of the dike which we assume is large in comparison to z_1 . If z_2 is just twice z_1 , it affects Q_z only 2%.

Simmons (ref. 18, equation 17) gives the surface heat-flow distribution as:

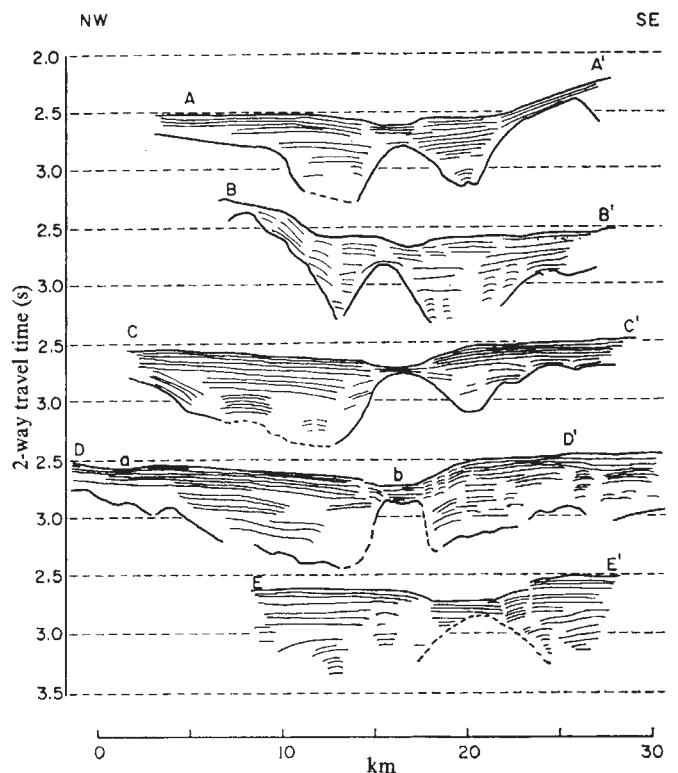
$$Q_z \big|_{z=0} = \frac{-KS}{2(\pi kt)^{1/2}} E(x, x_1, x_2) [\exp(-z_1^2/4kt) - \exp(-z_2^2/4kt)]$$

In this model, the same thermal properties are assumed for the dike as for the material into which it is intruded. We assume the thermal properties of sediments to be more realistic because the cooling is probably dominated by the insulating properties of the sediment. But the igneous body should be able to release heat faster, and should be younger than our solutions indicate. In Fig. 6 however, we show that both sediment and igneous rock thermal properties can be made to match the data. We have taken a conductivity, K , of 2×10^{-3} calorie $^{\circ}\text{C}^{-1} \text{s}^{-1} \text{cm}^{-1}$ ($0.83 \text{ W m}^{-1} \text{K}^{-1}$) and diffusivity, k , of $2.35 \times 10^{-3} \text{ cm}^2 \text{s}^{-1}$ to approximate a slightly compacted sediment column²⁰. Even greater values might be expected considering the probable metamorphism of the sediments directly above the intruded body. From the seismic reflection profile D-D' shown in Fig. 5, it seems that the anomaly is due to an intrusion

that is directly underneath the depression. From Fig. 5 the intrusion seems to be present and approximately the same shape on profiles A-A' through D-D' and is vague but present on E-E'. The intrusion seems to extend from one transform fault to the other. On profile D-D', closest to the profile of heat-flow stations, the depth to the top of the intrusion seems to be about 120–200 m. Figure 6 shows that using a depth of 200 m and removing the background heat flow, our anomaly is closely matched with a width of 1.3 km, and an age of 17,000 yr. For this model a maximum heat flow of 53 HFU would be reached at about 2,700 yr after the emplacement of the intrusion. The observed heat-flow maximum of 30 HFU is found twice for any model that assumes $z=200$ m, once at about 1,000 yr and again at 17,000 yr. The earlier time value seems unacceptable because it produces a very steep-sided anomaly as shown in Fig. 6, unlike the profile measured. If one assumes a depth to intrusion of 100 m, then an age of 18,000 yr very nearly models the observed anomaly. A greater depth to the intrusion is more difficult to model with thermal properties of sediments, although the thermal properties of the intrusive rock will fit the observed curve for a depth to intrusion of slightly greater than 500 m.

The seismic reflection profile indicates that the base of the intrusion may be 4 km in width, equivalent to about 65,000 yr of spreading at the half-rate of 30 mm yr^{-1} . It seems plausible to assume that the most recent intrusion is simply the latest in a continuing series of recent intrusions now totalling 4 km. If the most recent intrusion was emplaced into an older intrusion not at thermal equilibrium then our model would be less representative and much more recent ages of intrusion are possible. A 1.3 km wide intrusion would account for 21,000 yr of spreading. Since we have more than 42 m of sediment (2 m per 1,000 yr for 21,000 yr), we assume that the intrusion has always been covered. Hydrothermal cooling which might account for removal of about half the heat would be roughly equivalent to doubling the thermal conductivity (similar to the conductivity

Fig. 5 Line drawings of seismic reflection profiles. Line of profiles shown on Fig. 2. In profile D-D', a indicates a possible depression that is either just forming or is being filled and b indicates the area of the main heat-flow anomaly.



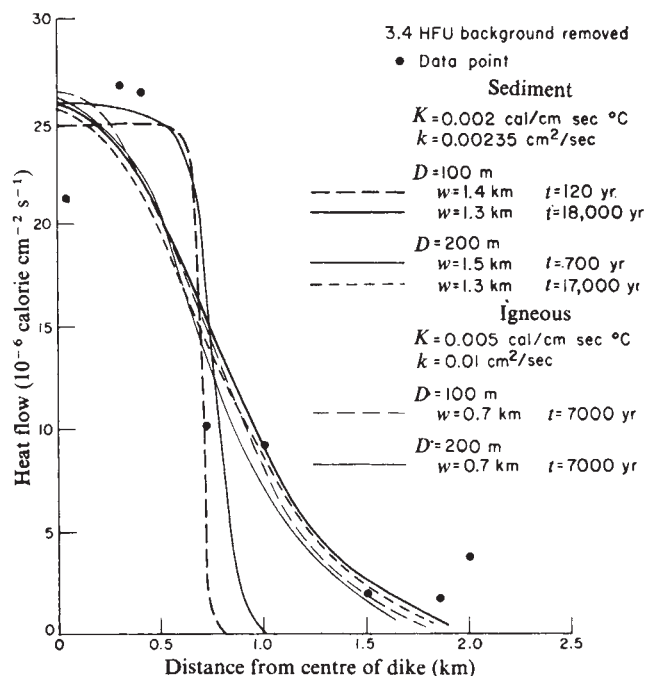


Fig. 6 Results of Simmon's¹⁸ model for heat-flow produced by a cooling intrusion.

of the intrusive rock). Although there are many variable parameters not defined by our data, the results from the model can be summarised as indicating an intrusion approximately 1.0 km wide, beneath 150 ± 100 m of sediments and less than 18,000 yr old.

Conclusions

Our findings allowed us to use heat-flow measurements to model a reasonably young intrusion, something that is rarely achieved. It does seem reasonably certain from our results that the gulf is not being opened by a continuing constant intrusion as is presumed at most oceanic spreading centres. Discontinuous and episodic intrusions into a thick sedimentary cover would explain the lack of magnetic anomalies in the gulf. Apparently the intrusion is continuous between transform faults and is believed contemporaneous.

The Gulf of California has an average sediment depth of

about 1 km (ref. 16). This, coupled with the high background heat flow, suggests that temperatures of 200 °C should generally be found near the sediment-rock interface. This is in addition to large areas where the thermal gradient is several times as high. If the upper regions of the basement are as porous and permeable as we believe, the Gulf of California would be one of the Earth's most important geothermal resources. This theory, though, can only be tested by drilling.

More measurements are needed in this area to trace the assumed lateral extent of the anomaly. Since the intrusion seems to be continuous between the transform faults, one would hope that the heat flow remains uniformly high in this region. Other sites in the Guaymas Basin and particularly the Farallon Basin to the south may be undergoing current intrusion and should be investigated.

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Three-dimensional model of purple membrane obtained by electron microscopy

R. Henderson & P. N. T. Unwin

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

A 7-Å resolution map of the purple membrane has been obtained by electron microscopy of tilted, unstained specimens. The protein in the membrane contains seven, closely packed, α -helical segments which extend roughly perpendicular to the plane of the membrane for most of its width. Lipid bilayer regions fill the spaces between the protein molecules.

THE purple membrane is a specialised part of the cell membrane of *Halobacterium halobium*¹. Oesterhelt and Stoekenius² have

shown that it functions *in vivo* as a light-driven hydrogen ion pump involved in photosynthesis. It contains identical protein molecules of molecular weight 26,000, which make up 75% of the total mass, and lipid which makes up the remaining 25% (ref. 3). Retinal, covalently linked to each protein molecule in a 1:1 ratio is responsible for the characteristic purple colour³. These components together form an extremely regular two-dimensional array⁴.

We have studied the purple membrane by electron microscopy using a method for determining the projected structures of unstained crystalline specimens⁵. By applying the method to tilted specimens, and using the principles put forward by

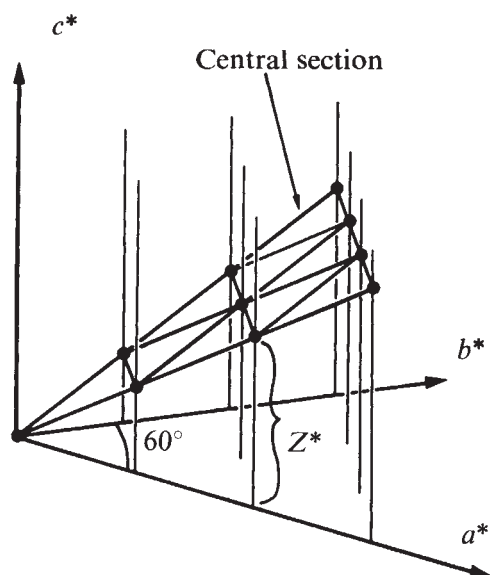


Fig. 1 Part of the three-dimensional reciprocal lattice showing the geometry of the lattice lines in the hexagonal space group P3. a^* , b^* and c^* are the reciprocal lattice vectors. a^* and b^* lie in, and c^* is perpendicular to the plane of the membrane. A central section which is perpendicular to the incident electron beam has been drawn through the lattice. The intersection of this central section with the reciprocal lattice is determined by the angle of tilt and the axis about which the membrane is tilted. Individual diffraction patterns and micrographs provide the amplitudes and phases in this section at the points shown. z^* represents the coordinate along the c^* direction of one of the points. The angle of tilt was measured to within 2° for each of the specially modified, tilted specimen holders, and the direction of the tilt axis on the photographic plate was established during operation of the microscope. However, estimates based on the geometry of the spacings of the lattice points (for high tilt angles), the variation of the degree of underfocus across the plate (for low tilt angles), and least squares refinement against data obtained at high tilt angles (for diffraction patterns) provided more accurate figures which were used in the calculation. The accuracy of measurement of both the amplitudes and phases depended on having sharp lattice lines. We therefore took care to ensure that, on the microscope grid, the membranes remained coherently ordered and flat to within $1/5^\circ$.

De Rosier and Klug⁶ for the combination of such two-dimensional views, we have obtained a three-dimensional map of the membrane at 7 Å resolution. The map reveals the location of the protein and lipid components, the arrangement of the polypeptide chains within each protein molecule, and the relationship of the protein molecules in the lattice.

Electron microscopy and diffraction

The purple membrane was prepared under normal conditions from cultures of *H. halobium*³ and applied to the microscope grid in the presence of 0.5% glucose. The purified membranes are mostly oval sheets up to 1.0 µm in diameter and about 45 Å thick^{4,7}. The array of molecules making up these sheets is accurately described⁷ as an almost perfect crystal of space group P3 ($a = 62$ Å) with a thickness of one unit cell only in the direction of the c axis. A single membrane thus contains up to 40,000 unit cells; that is 120,000 protein molecules (three per unit cell).

These large periodic arrays from which electron diffraction patterns and defocused bright field micrographs are recorded⁵ enable us to overcome the principal problem normally associated with high resolution electron microscopy of unstained biological materials; that is, sensitivity to electron damage⁸. Only a small number of electrons can pass through each unit cell before it is destroyed, but because of the large number of unit cells, the information in the diffraction patterns and micrographs is sufficient to provide a picture of the average

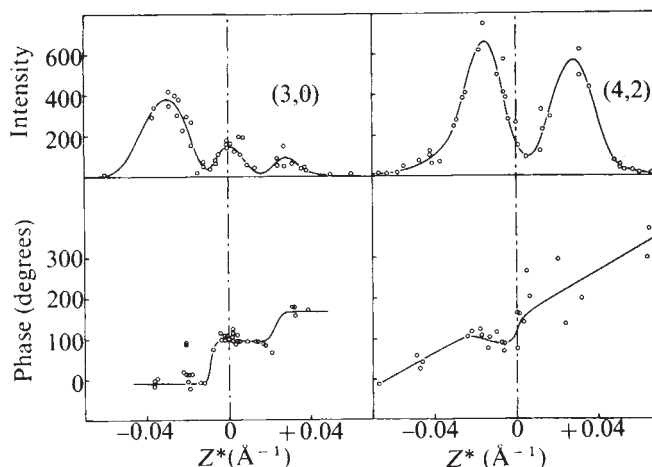
unit cell. The micrographs recorded with such low doses of electrons appear featureless, since the statistical fluctuation in the number of electrons striking the plate is large compared with the weak phase contrast ($<1\%$) produced by defocusing. As a result, analysis of each micrograph by densitometry and computer processing⁵ is required to combine the information from individual unit cells.

Solution of the three-dimensional structure of the purple membrane requires the determination of the amplitudes and phases in three dimensions of the Fourier terms into which it can be analysed. The diffraction pattern or Fourier transform of the membrane is not a three-dimensional lattice of points as is the case with a normal crystal, but since it is only one unit cell thick, a two-dimensional lattice of lines which are continuous in the direction of c^* (that is perpendicular to the membrane). A single electron diffraction experiment therefore gives rise to a two-dimensional pattern of spots which correspond in their intensities to the square of the amplitudes of the transform along a central section through the three dimensional transform of the membrane (Fig. 1). Similarly, an electron micrograph contains information about the phases (and about the amplitudes, but less accurately than does the diffraction pattern) in the central section. Previously⁵, we were able to determine the projected structures of both the purple membrane and thin catalase crystals from the amplitudes (obtained by electron diffraction) and phases (by Fourier analysis of micrographs) along the single central section through the transform parallel to the plane of the object.

Here, we have collected data similar to those obtained previously but from 15 diffraction patterns and 18 micrographs (actually pairs of micrographs, see ref. 5) of membranes tilted at angles from 0° to 57° to the incident electron beam, to determine the amplitudes and phases at a number of points along each of the lattice lines. These data provided estimates of the continuous variation of the transform along the lines. By sampling the continuous curves at appropriate intervals, it was then possible to calculate the structure as if for a three-dimensional crystal with some arbitrary c -axis dimension greater than the membrane thickness. This method of combining two-dimensional images was proposed originally by De Rosier and Klug⁶ for a general object, although in this case we have made use of electron diffraction patterns as well.

The diffraction patterns provided 1,800 independent intensity measurements which were combined to give continuous curves, such as those shown in Fig. 2, for each of the 36 crystallographically independent lattice lines out to a resolution of 7 Å.

Fig. 2 Values of intensity and phase along two lattice lines, obtained respectively from the diffraction patterns and images using the geometry shown in Fig. 1. Smooth curves were drawn through the points to give reliable intensities and phases at suitably fine sampling intervals (0.01 Å^{-1}) in z^* . These values were used to compute the three-dimensional structure.



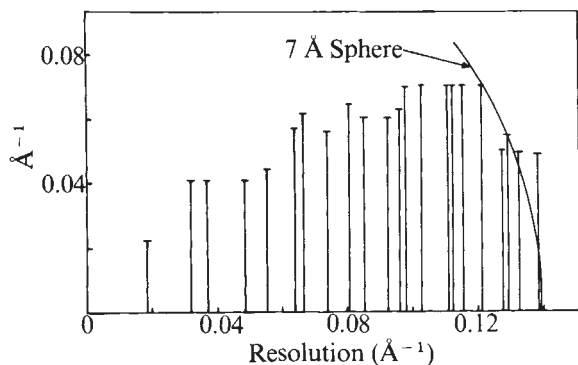


Fig. 3 Schematic diagram showing the region of reciprocal space contributing to the three-dimensional Fourier map. Part of the boundary of the 7 Å sphere is indicated. The region shown, which includes 63% of the total volume of this sphere, includes almost all the strong diffraction peaks to 7 Å, with the principal exception of the axial (0,0) lattice line (but see text).

Because of the distribution of intensity in the transform⁷, due to real features in the structure, very little diffracted intensity (none greater than 1/50th of the strongest intensity) occurred at values of z^* greater than 0.08 Å⁻¹ for any lattice line.

With the electron micrographs compensation for the effects of defocus was rather more complicated than in the case of untitled specimens⁵. Since the membranes we studied were at least 5,000 Å in diameter, different parts of the membrane in a tilted specimen were situated at significantly different distances from the objective lens. As a result, the phase contrast transfer function, which depends on the degree of underfocus of this lens, varied across the image. The correction of the contrast of the image could not be made after transforming the image by altering the phases of the Fourier components of the image, as was done previously, but instead had to be carried out at an earlier stage, so that different parts of the image where the contrast is positive or negative could be made to combine constructively, and thereby yield, on transformation, the correct phases for the object. This procedure was conveniently carried out between the execution of the first and second dimensions of the Fourier transformation having ensured that the images were densitometered parallel to the tilt axis. The contrast along each scan line could then be corrected by multiplying the one-dimensionally transformed data by the contrast transfer function, $-2 \sin x$ (see ref. 5) for each reflection, so that the computed phases had the correct sign for the object, and gave the maximum signal-to-noise ratio. The phases were then combined image by image after refining the phase origin of each image so that the best agreement of its phases with all previously accumulated phases was obtained. By starting with the untitled data with the phase origin at one of the threefold positions, and adding the phases from images with smaller tilt angles first, the maximum accuracy in combining the phases was ensured. This procedure also meant that the phase origin for the combined three-dimensional data ended roughly in the middle of the membrane on one of the threefold axes. The average phase error in a single measurement estimated from the differences between those of the 1,000 independently recorded phases having similar values of z^* was less than 20°, as found previously⁵. As with the intensity data, continuous curves could be drawn through these phase measurements (Fig. 2).

The intensity and phase curves were sampled at intervals of 0.01 Å⁻¹ to provide Fourier terms from which to calculate the three-dimensional map. This sampling interval is more than sufficiently fine to ensure an accurate representation of a structure only 45 Å thick.

Three-dimensional potential map

The map (Fig. 4) was a Fourier synthesis of 365 crystallographically independent terms which extended to a resolution

of 7 Å in the plane of the membrane, but which included only terms with spacings greater than 14 Å perpendicular to it. There were two reasons for the asymmetrical distribution of Fourier terms. First, the distribution of intensity in the transform of the membrane is such that the diffraction beyond spacings of 7 Å in the plane of the membrane, and about 20 Å perpendicular to it, is weak and therefore difficult to measure with the limited total diffraction available from membranes only 1 μm in diameter. The average intensity in these regions by X-ray diffraction⁷ is 7–10 times lower than the in-plane diffraction near 10 Å, and by electron diffraction we find no intensity greater than 1/50th of the strongest diffraction at values of z^* greater than 0.080 Å⁻¹. Second, the use of tilt angles up to some limiting angle, in this case 57°, meant that a conical region of reciprocal space was unmeasured. The volume of reciprocal space finally included is shown schematically in Fig. 3. Although a considerable volume of the 7 Å sphere is missing (37%) the effect of its inclusion in the map would be very small since the X-ray diffraction pattern indicates that the amplitudes of the Fourier components in the missing regions are small. Therefore, since the map includes all the strong terms present to 7 Å resolution, it provides an accurate representation of the dominating features of the structure to this resolution.

As a further check on the effect of leaving out some of the near axial reflections, a second map was calculated which included the profile diffraction determined by X rays. The amplitudes were obtained from the published X-ray profile

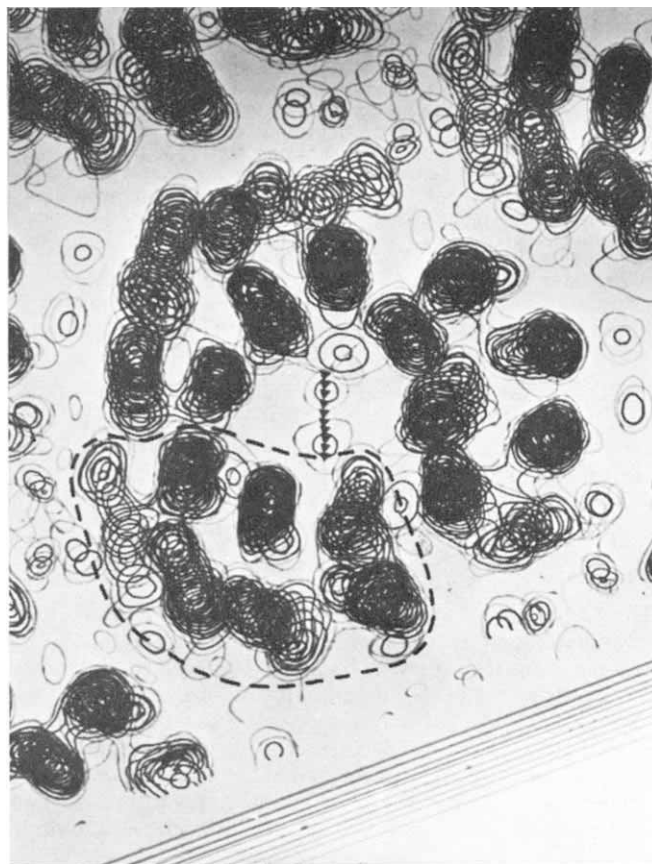


Fig. 4 Part of the three-dimensional potential map showing a region 50 Å thick spanning the membrane. The sections above and below this region contain no features higher than one contour level. The absolute hand is determined by the known direction of specimen tilt. The view shows protein molecules grouped around one of the threefold axes. The probable boundary of one of them is indicated by the broken line. The angle of view is such that the α helices furthest from the threefold axis partly overlap in the protein molecule outlined, but are seen almost end-on in the one on the right.

of Blaurock and Stoeckenius⁴ by sampling at $0.01 \text{ \AA}^{-1}$ intervals, and scaled to our data by comparing the profile intensity with that of the (1,1) ring in an unoriented X-ray powder pattern. The phases were assumed to be those of a symmetrical membrane⁴ with the origin in the middle. The inclusion of the Fourier terms out to $0.05 \text{ \AA}^{-1}$ made little difference to the map but, as would be expected, slightly raised the overall level of the contours on each side of the membrane (by about one contour level). The axial (0,0) line is by far the strongest region not included in our map, so clearly the effect of excluding the remaining unmeasured volume of reciprocal space is small.

Since the amplitudes and phases used to calculate the present map are derived from the scattering of electrons, the physical quantity which it represents is, strictly, the electrical potential inside the membrane. At this resolution, however, the potential is roughly proportional both to the electron density, as would be found for instance by X-ray analysis, and to the atomic number. High density features therefore correspond to the presence of denser groupings of atoms.

Structure of the membrane

The appearance of the map is dominated by numerous rod-shaped features aligned perpendicular to the plane of the membrane. There are seven rods in each asymmetric unit of the crystal and they are packed $10\text{--}12 \text{ \AA}$ apart. Adjacent rods are slightly inclined to one another at various angles from 0° to 20° and they are all roughly $35\text{--}40 \text{ \AA}$ long. In view of the existence^{7,9} in the X-ray diffraction pattern of strong axial reflections at $1.5 \text{ \AA}$ and $5 \text{ \AA}$ which are characteristic of the α -helix, there is little doubt that the seven rod-shaped features in the map are α -helices which extend perpendicular to the membrane for most of its width. Since the protein has a molecular weight of 26,000 (ref. 3), it follows that the α -helices make up 70–80% of the polypeptide. The connectivity of the helices, however, cannot be discerned unambiguously at this resolution.

The tentative boundary of an individual protein molecule indicated in Fig. 4 is the one most likely to be correct since it surrounds regions of the protein with maximum connectivity and it is this part of the map which we have used to make the model shown in Fig. 5. The overall dimensions of the protein in our model are $25 \times 35 \times 45 \text{ \AA}$, with the longest dimension perpendicular to the plane of the membrane and parallel to the helices. The three protein molecules which are most intimately in contact are grouped round the threefold axis in an interesting manner. Three of the seven α helices in each protein molecule are nearer the centre of the group giving an inner ring of nine helices which are all $10 \text{ \AA} (\pm 1)$ apart and in contact with one another. The other four helices in each protein combine to make an outer ring of twelve which surround the inner nine. The outer helices are all slightly more inclined to the perpendicular than the inner ones, and are not all in contact. The three most strongly tilted of these helices were not resolved in projection⁵, since they partly superimpose when viewed perpendicular to the plane of the membrane. The direction of tilting of the outer helices is consistent with the interlocking of the amino acid side chains from adjacent helices—that is the structure is a left-handed supercoil, as expected for a right-handed α helix¹⁰.

In the space in the middle of the ring of nine helices, we presume that a small number of lipid molecules is arranged in the classical bilayer configuration. This space has a diameter of about $20 \text{ \AA}$; it is not a hole filled with solvent since a difference map using X-ray intensities from wet versus dry membranes is featureless. Evidence obtained from another difference map shows that UO_2^{+2} ions can bind to this region of the membrane, again suggesting that the space is filled with lipid. Similar arguments that a lipid bilayer must be present apply to the rather even density regions which make up the remainder of the membrane structure, filling up the space between the clusters of three protein molecules.

At this resolution we cannot detect the covalently linked

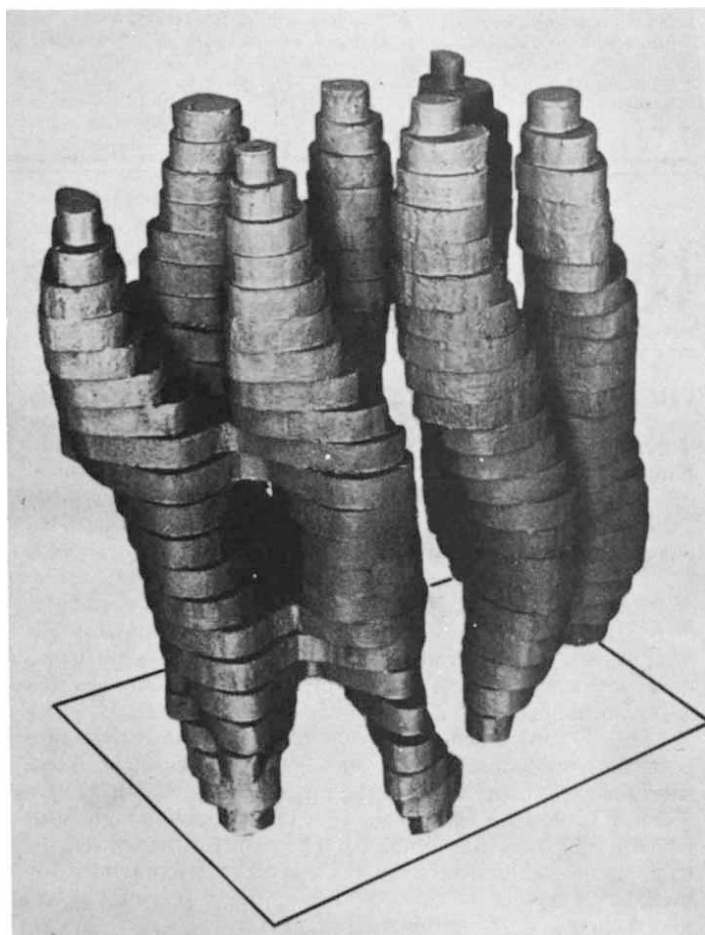


Fig. 5 A model of a single protein molecule in the purple membrane, viewed roughly parallel to the plane of the membrane. The top and bottom of the model correspond to the parts of the protein in contact with the solvent, the rest being in contact with lipid. The most strongly tilted α helices are in the foreground.

retinal molecule, which is thought, from measurements of the dichroism at 560 nm in oriented specimens⁴, to be aligned parallel to the plane of the membrane—that is, perpendicular to the helices.

The structure of the purple membrane protein and the way it combines with lipid to form a two-dimensional mosaic in which the protein and lipid pack neatly side by side, each with a thickness of about $45 \text{ \AA}$, gives experimental support to recent concepts^{11,12} of membrane structure based on less direct evidence. In particular, the protein is globular, is almost certainly exposed on both sides of the membrane, and is surrounded by lipids which are arranged in separate areas with a bilayer configuration. The purple membrane thus seems to provide a simple example of an 'intrinsic' membrane protein, a class of structure to which many molecular pumps and channels must belong. We would not be surprised if the simple arrangement of helices found here also occurs in some of these other intrinsic membrane proteins.

A full account of this work will be published elsewhere. We thank Dr J. Pilkington and the Royal Greenwich Observatory for help with and facilities for the densitometry of the micrographs, Chris Raeburn for modifying the specimen holders and Drs L. Amos, T. Horsnell, R. Ladner and T. Takano for computer programs. We also thank our colleagues in this laboratory, particularly Dr A. Klug, for comments on the manuscript.

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letters to nature

Observations of X rays from near NGC6440

ON nine separate occasions between October 27, 1971, and January 6, 1973, the MIT instrument aboard OSO-7 scanned the region of sky within about 10° of the galactic centre. Subsequent analysis of this data, in the form of all-sky maps, led to the discovery of several previously unreported X-ray sources¹. The most intense of these, MX1746–20, was found at $\alpha = 17^h 46.0^m$, $\delta = -20^\circ 22.2'$ (1950.0) or, in galactic coordinates $l^{\text{II}} = 7.73^\circ$, $b^{\text{II}} = 3.76^\circ$. The 90% confidence contour for this position is a circle of radius approximately 0.26° . This uncertainty includes an estimate of the probable aspect error as well as statistical uncertainty.

The MIT instrument² consisted of two banks of five proportional counters sensitive from 1–60 keV. The mean intensities for each of the scans of the position of MX1746–20 are given in Table 1 for each of the four proportional counters for which useful data were obtained. The data for the low-energy counters are omitted in some cases because of contamination by solar X rays or because of the failure of one of the two lowest energy counters after August 2, 1972.

MX1746–20 is a highly variable X-ray source on time scales of months. Variations of at least a factor of 20 are observed. We have seen only weak evidence for variation on shorter time

of globular clusters as a function of angular separation from the galactic centre, we determined the probability of a chance coincidence of MX1746–20 with any globular cluster to be about 1%.

The distance to NGC6440 has not been well established although it is likely to be found close to the galactic centre. This hypothesis is supported by the galactic coordinates of the source and by its late spectral type (G5) which is believed to be typical of clusters around the centre of the Galaxy⁴. Assuming a distance of 10 kpc to MX1746–20, we derive a peak 1–10 keV X-ray luminosity of 3.4×10^{37} erg s⁻¹.

Like the previously discovered globular cluster X-ray sources^{3,5,6}, MX1746–20 is highly variable and its counterpart, NGC6440, is concentrated (concentration class V). This latter characteristic of the cluster adds credence to those theories of globular cluster X-ray emission which require high central densities to encourage the formation of binary systems (ref. 7 and unpublished work by A. C. Fabian *et al.*), or to provide a high enough escape velocity to cause a gas accretion rate sufficient to power the observed X-ray production from the vicinity of massive black holes (ref. 8 and J. Silk and J. Arons, unpublished).

Since all of the cluster X-ray sources discovered thus far are variable³, it may be that many more remain to be found. At

Table 1 X-ray flux of MX1746–20

Date	Intensity* (counts s ⁻¹)			
	1.0–1.5 keV	1–6 keV	3–10 keV	15–40 keV
October 27–30, 1971	1.0 ± 1.2	0.5 ± 1.0	0.8 ± 0.7	–0.1 ± 1.0
December 02–06, 1971	—	—	2.5 ± 1.0	0.3 ± 0.3
January 01–06, 1972	1.9 ± 0.7	16.4 ± 0.7	9.7 ± 0.4	0.3 ± 0.4
June 01–04, 1972	1.4 ± 2.1	–0.5 ± 2.1	0.9 ± 1.5	0.1 ± 1.9
June 30–July 09, 1972	–0.2 ± 0.2	1.6 ± 1.0	1.1 ± 0.4	0.4 ± 0.3
August 28–31, 1972	—	3.2 ± 1.2	1.8 ± 0.5	0.4 ± 0.3
September 14–20, 1972	—	4.2 ± 1.5	2.9 ± 0.7	0.7 ± 0.4
December 03–09, 1972	—	—	0.9 ± 0.4	0.4 ± 0.2
January 02–06, 1973	0.2 ± 0.5	–0.7 ± 0.8	–0.4 ± 0.3	0.4 ± 0.4

*The observed intensities may be converted to photon fluxes by the approximate formula $\Phi = \gamma/I$ where Φ = flux in photons cm⁻² s⁻¹ keV⁻¹, I = intensity in counts s⁻¹, and $\gamma = 0.15$ (1.0–1.5 keV), 0.0069 (1–6 keV), 0.0045 (3–10 keV), 0.0013 (15–40 keV).

scales although the statistical precision of our data hampers our ability to observe fluctuations less than about 5 counts s⁻¹ on time scales shorter than a day.

We were able to fit parameters describing the spectrum of MX1746–20 for the observations of January 1972. Because of the small number of data points available, however, we were unable to determine the nature of the spectrum unambiguously. For a trial thermal bremsstrahlung spectrum, a temperature of 7.7 ± 3.5 keV and a hydrogen column density of $1.1^{+1.0}_{-0.8} \times 10^{22}$ atoms cm⁻² were determined. For a power law spectrum, we found a photon spectral index of $2.1^{+0.8}_{-0.5}$ and a hydrogen column density of $5.3^{+3.0}_{-2.0} \times 10^{22}$ atom cm⁻².

A search of star catalogues and catalogues of interesting objects yielded only one likely candidate in the MX1746–20 error circle—the globular cluster NGC6440. NGC6440 is by far the most prominent object in the error circle and the only object brighter than $m_v = 10$, in spite of the high density of stars in the region. Using an empirical formula³ for the density

least two of the sources, MX0513–40 and MX1746–20, vary so greatly that they frequently fall below the level of detectability of the MIT OSO-7 instrument. We suggest that current and future satellite experiments, particularly those with high sensitivity, observe concentrated globular clusters from time to time since it is possible that a large fraction of them emit X rays on occasion.

T. H. MARKERT
 D. E. BACKMAN
 C. R. CANIZARES
 G. W. CLARK
 A. M. LEVINE

Center for Space Research,
 Massachusetts Institute of Technology,
 Cambridge, Massachusetts 02139

Received June 30; accepted July 16, 1975.

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Abell 478 and X-ray source 3U0405+10

THREE sightings of the faint X-ray source 3U0405+10 with the Ariel V sky survey instrument have produced an improved position, with the error box now containing Abell 478. This distance class 6 Abell cluster is the most distant X-ray source yet detected. It has a (2–20 keV) X-ray luminosity of $(5.4 \pm 1.1) \times 10^{45} \text{ erg s}^{-1}$.

The instrument detected X-ray emission from 3U0405+10 on October 28, 1974, with both high (2.4–19.8 keV) and low (1.2–5.8 keV) energy detector systems. Combining these two lines of position with a later cross scan, from the HE system on March 17, 1975, the revised position of 3U0405+10 has been derived; correcting the Uhuru count rate to the new position gives a slightly higher rate statistically consistent with the Ariel V results.

The sky survey experiment scans a strip of sky around the spin plane of the satellite. The response of the collimators is designed to produce long, thin lines of position for the sources detected. The high and low energy systems are canted by $\pm 25^\circ$ respectively to the spin axis direction so that a source seen in both systems will have an error box limited to the crossing of the two lines of position. The FWHM of the collimators is 0.73° in the spin plane and 10.6° along the line of position. The width of each line of position has been taken as the FWHM of the collimator divided by the significance of the signal above the background. This corresponds approximately to a 90% confidence that the source lies within the boundaries of any line. Table 1 lists the observations and Fig. 1 shows the new error box, together with that from the earlier Uhuru observations.

The refined error box now contains the cluster of galaxies Abell 478 (Fig. 2) which was just outside the 3U error box¹. Furthermore, the area of the error box is now reduced to 0.27 square degrees from our data alone; combined with the

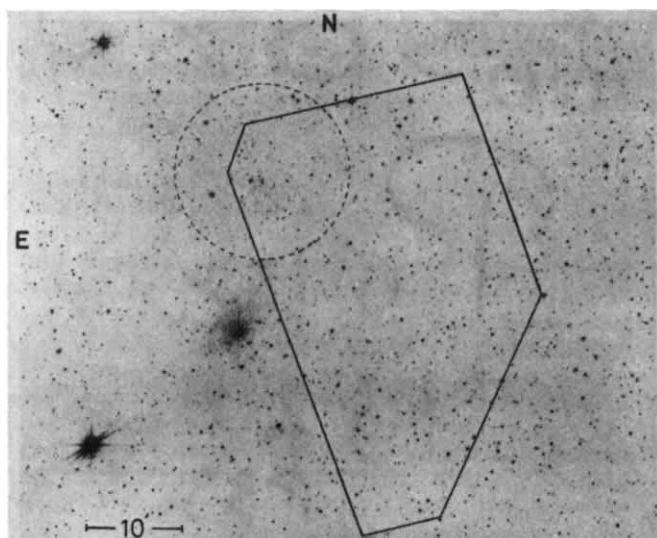


Fig. 2 Ariel V error box superimposed on Palomar Sky Survey photograph. The brightest galaxy in the A478 cluster is clearly seen in the north-east corner of the box; the circle indicates the approximate extent of the cluster. (Photograph copyright National Geographic Society–Palomar Observatory Sky Survey.)

Uhuru observations this is further reduced to ~ 0.1 square degrees. We regard this improved location as confirming the association of A478 with the X-ray source 3U0405+10. From a positional correlation of all the rich clusters in the Abell catalogue with the actual 3U sources and with similar distributions of sources from 20 'fake' catalogues, J. N. Bahcall and N. A. Bahcall (see ref. 2) have concluded that there is no good evidence for the association of any of the most distant (distance class 5 or 6) Abell clusters with X-ray sources in the 3U catalogue. Specifically, their analysis for 3U sources with error boxes ≤ 5 square degrees produced four associations with distance class 5 and 6, whereas the 'fake' source catalogue gave 2.7 ± 1.5 associations. The fivefold reduction in the error box area of 3U0405+10 greatly increases the probability of a physical association with A478, a rough estimate based on the number density of clusters and of 3U sources now giving a probability in excess of 80%.

Taking Abell's redshift value of 0.18 for distance class 6 clusters³ and a Hubble constant of $60 \text{ km s}^{-1} \text{ Mpc}^{-1}$ gives a distance of 900 Mpc. If we take the mean energy of our measured flux to be 4.5 keV (as it would be for a spectrum like that of the Crab source) then the two high energy measurements, combined, give an X-ray luminosity (2.4–19.8 keV) of $(5.4 \pm 1.1) \times 10^{45} \text{ erg s}^{-1}$. This makes A478 the most distant X-ray source

Fig. 1 Three intersecting lines of position from presently reported Ariel V observations give error box shown by thick line. Also shown are previous Uhuru position of 3U0405+10 and centre of A478 (1950 coordinates).

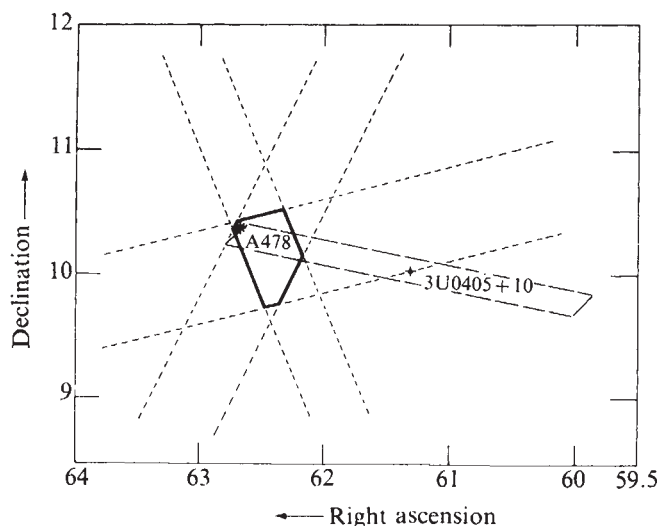


Table 1 Observations of 3U0405+10 with Ariel V sky survey

Date	Energy band (keV)	No. of orbits summed	Incident flux* (erg cm ⁻² s ⁻¹)
October 28, 1974	2.4–19.8	18	$(4.6 \pm 1.2) \times 10^{-11}$
October 28, 1974	1.2–5.8	18	$(6.6 \pm 2.3) \times 10^{-11}$
March 17, 1975	2.4–19.8	27	$(6.9 \pm 2.8) \times 10^{-11}$

* Assuming spectrum like that of Crab source.

yet detected and also the most luminous of all the X-ray cluster sources. Interest in the apparent relationship between the X-ray luminosity of clusters and the velocity dispersion of their brighter individual galaxies (for example, ref. 4) makes it of considerable importance to measure the value for A478, which is inferred, to lie in the range $4,000\text{--}7,000 \text{ km s}^{-1}$.

We thank Drs J. Bahcall and N. Bahcall for making available the results of their positional correlation studies, Dr M. V. Penston

for providing the overlay of Fig. 2 and the Hale Observatories for permission to reproduce the Palomar chart.

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M. ELVIS
B. A. COOKE
K. A. POUNDS
M. J. L. TURNER

*X-ray Astronomy Group,
Department of Physics,
University of Leicester,
Leicester, UK*

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Galactic X-ray sources and the ionospheric D region

SEVERAL authors (see refs 1-5 for example) have studied the possible influence of galactic point X-ray sources in the ionospheric D region. The observed variabilities in phase and amplitude of very low frequency (v.l.f.) night-time propagation have been attributed to different ionisation sources, but the relationship between the appearance of strong X-ray sources in the sky and the recorded v.l.f. phase and intensity variations has been emphasised. Particularly important are the effects of Sco X-1, Cen X-2 and Cen X-4.

Ion production rates attributable to galactic point X-ray sources have been evaluated², considering a constant photoionisation equal to $1/0.032 \text{ keV}^{-1}$ and energies of up to 10 keV. These calculations do not include all of the energy ranges available in the measured spectra. Therefore, the results obtained suggest that the contribution of point X-ray sources to the total ion production of the D region is small.

We calculate here theoretical ionisation rates which include energies up to 50 keV, non-constant photoionisation yields⁶ and the latest available measured spectra. The total electron production rate attributable to a celestial X-ray source is given by:

$$Q(z) = \int_{E_0}^{E_{\max}} q(E, z) dE$$

where

$$q(E, z) = I(E, z) \sum_i \gamma_i(E) \sigma_i(E) n_i(z)$$

and

$$I(E, z) = I_0(E) \exp[\sec\theta \sum_i \sigma_i(E) \int_z^\infty n_i(z) dz]$$

where, $I_0(E)$ is the intensity of the emitted X rays at the top of the atmosphere; suffix i indicates the neutral constituent species considered (N_2 , O_2 and O); $n_i(z)$ is the number density of neutral atmospheric constituent i at altitude z ; $\sigma_i(E)$ the absorption cross section of neutral atmospheric constituent i for electromagnetic radiation over an energy range E ; and $\gamma(E)$ is the photoionisation yield, that is, the number of electron ion pairs formed per photon with energy E absorbed by a neutral atmospheric constituent i .

Figure 1 shows the ionisation rate attributable to Tau X-1 (ref. 7), Sco X-1 at 0° , 30° and 45° for normal conditions⁸ where $I_0(E) = 110 \exp(E/4.3)$, and for flare conditions⁹ together with the ion production attributable to other minor point X-ray sources in the southern sky¹⁰. It is important to

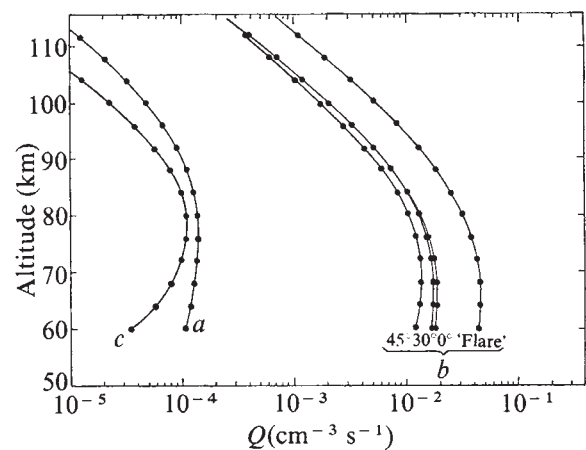


Fig. 1 Rates of ionisation production from: a, Tau X-1; b, Sco X-1; c, other minor sources in the Southern Hemisphere sky.

note that the profiles shown differ considerably from those given in the publications mentioned.

Figure 2 shows the ionisation rates attributable to major night-time ionisation sources: Lyman β , considering the flux given by Young *et al.*¹¹; scattered Lyman α calculated using the NO density distribution given by Meira¹², and the flux given by Winter *et al.*¹³; cosmic rays¹⁴ and the contribution of a diffuse galactic X-ray background⁶.

The contribution of Sco X-1 to the night-time ion production, is significant compared with the other major night-time sources, but the effect of the minor X-ray sources included can be neglected. Therefore, these results support claims that there is a Scorpius X-1 effect on the v.l.f. signal propagation records.

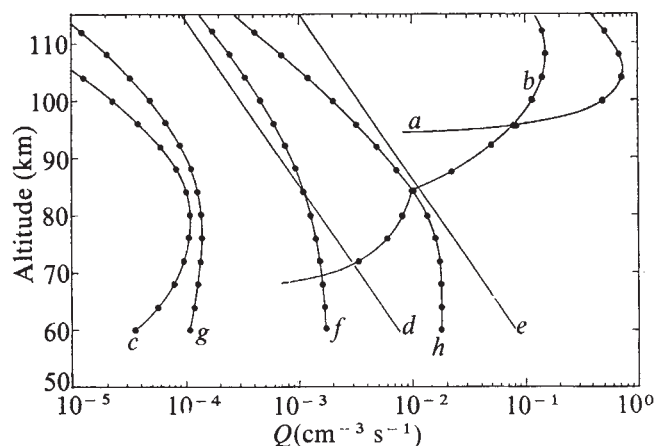


Fig. 2 Rates of ionisation production from major night-time ionisation sources: a, Lyman- β radiation; b, scattered Lyman- α radiation; c, stellar X-ray sources; d, galactic cosmic rays ($\gamma_m = 0^\circ$); e, galactic cosmic rays ($\gamma_m = 55^\circ$); f, diffuse galactic X-ray background; g, Tau X-1; h, Sco X-1.

Similar conclusions could be drawn for Cen X-2 (ref. 15) and Cen X-4 (ref. 16) since it has been shown that their spectral shape and intensity are comparable with those of Sco X-1.

This work has been carried out under the direction of Professor Sandro Radicella at the Aeronautics Department, Universidad Nacional de la Plata.

HAYDEE KARSZENBAUM
DOMINGO A. GAGLIARDINI

*Aeronomy Group,
Engineering Department,
Universidad Nacional de La Plata, and
Comisión Nacional de Estudios Geo-Heliósficos,
Corrientes 640, Piso 11,
Buenos Aires, Argentina*

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Geomagnetic effect associated with X-ray flare from Sco X-1

THE effect on the night-time lower ionosphere of X radiation from celestial sources is a topic of some controversy¹⁻⁴. An X-ray flare from Sco X-1 has been shown to have a daytime effect on the ionosphere⁵ and it has also been reported⁶ that celestial X-ray sources have a nocturnal effect on the lower ionosphere in certain conditions. Galactic X-ray ionisation has been shown⁷⁻⁹ to affect night-time geomagnetic field components.

We have examined the possibility that an X-ray flare from Sco X-1, detected in the course of a balloon flight on October 15, 1967 at Mildura (34° 12'S; 142° 6'E)¹⁰, had a daytime geomagnetic effect. We observed a conspicuous perturbation in the form of an increase in the horizontal component of the magnetic field observed at the equatorial station at Kodaikanal (10° 14'N, 77° 28'E).

On October 15, 1967, Sco X-1 flared up at about 0700 UT, reaching a maximum in less than 10 min and then decreasing during the next 20 min (Fig. 1). The perturbation in the horizontal component of the magnetic field started around 0715 UT, and rose to a maximum at 0725 UT (an increase in the *H* component of about 14 γ), about 16 min after the flare had reached a maximum at around 0710 UT. Unfortunately, a small optical solar flare occurred about 15 min before the Sco X-1 flare, during the period 0647-0710 UT, with a maximum at 0650 UT.

In view of this, we have examined the possibility that the observed geomagnetic effect was caused by this solar flare, using the available solar X-ray flux data for this event. The procedure followed is similar to that adopted by Ramanamurthy et al.⁵. Solar X-ray flux data show a small burst in the 8-12 Å band during the period 0640-0713 UT but it is considered unlikely that it produced a solar flare effect (SFE), (geomagnetic crochet) as its amplitude is below the threshold value. Van Allen's data from Explorer 35 show a flare (with a flux enhancement of 2.3) which began at about 0640 UT, reached a maximum at about 0653 UT and ended at about 0730 UT. An earlier study made by us showed that the relaxation time of the SFE (geomagnetic crochet) with reference to the solar X-ray flare (1-20 Å) ranges from -10 to +22 min (with an average value of 4.0 min for the 1-8 Å band and 1.4 min for the 8-20 Å band). Thus, the observed time lag of about 33 min between the solar X-ray flare (2-12 Å) maximum and that of the observed geomagnetic effect is well outside the range of relaxation times for geomagnetic solar flare effects (SFE or crochet events).

The possible daytime geomagnetic effect of Sco X-1 is considered more interesting than the nocturnal effect reported earlier, as during the day the rate of ion production resulting from the Sco X-1 X-ray flux has to compete with the rate resulting from the solar X-ray flux and L_{α} besides that resulting from galactic cosmic rays. An estimation of the production rates

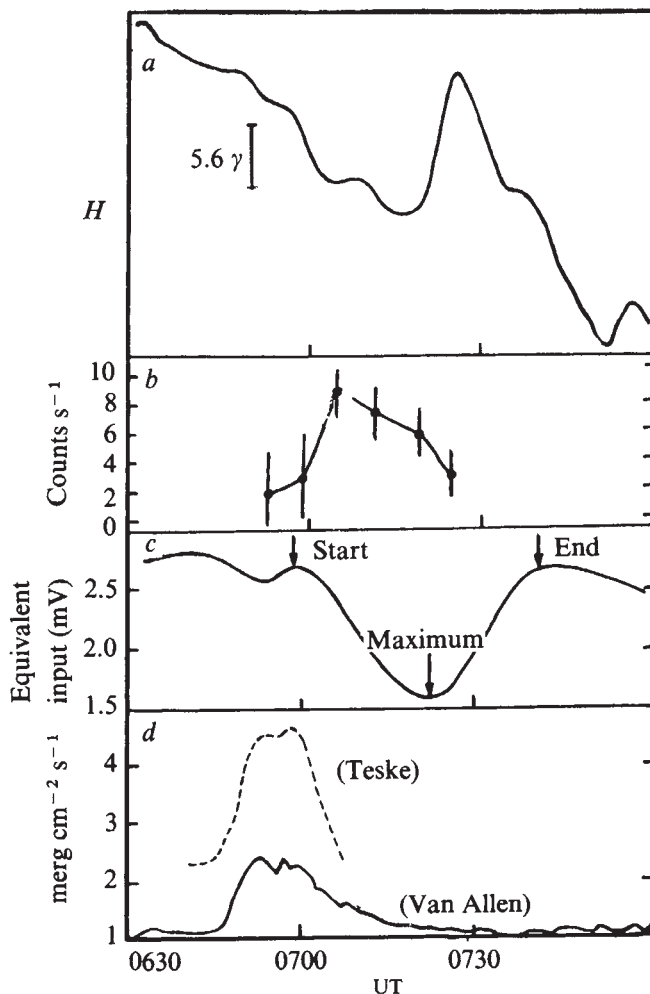


Fig. 1 Kodaikanal, October 15, 1967; temporal variations in: a, the horizontal component, *H* of the magnetic field at Kodaikanal; b, 20-30 keV X-ray counts from Sco XR-1 (ref. 10); c, the field strength (164 kHz) over the Tashkent-Delhi path (after Ramanamurthy et al.⁵); d, the solar X-ray flux 2-12 Å (Van Allen) and 8-12 Å (Teske), (after Ramanamurthy et al.⁵).

attributable to Sco X-1, L_{α} and galactic cosmic rays indicates that the rates of production attributable to the X-ray source are comparable with those attributable to cosmic rays and L_{α} at levels below about 70 km (ref. 5). So the observed geomagnetic effect could be attributable to X-ray flux enhancement (1-20 Å), as during an SFE (crochet) the region of enhanced conductivity lies in the D-region¹¹⁻¹⁴.

A sudden impulse (SI) was reported on October 15, 1967 at 0724 UT from two stations, SO and HL and an SSC was reported from station SU (Solar-Geophysical Data ESSA/NOAA). In view of the circumstantial evidence presented here, however, we do not consider the observed geomagnetic perturbation at Kodaikanal as an SI or an SSC but as a genuine effect of the X-ray flare from Sco X-1.

J. HANUMATH SASTRI

B. SURYANARAYANA MURTHY

Indian Institute of Astrophysics,
Kodaikanal 624103,
India

Received April 14; accepted June 3, 1975.

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Ghost echoes on the Earth-Moon path

ON July 7, 1974 while using a Moon Bounce technique on 1,296 MHz I observed the appearance of strange, delayed echoes. My equipment consists of a parabolic antenna 26 feet in diameter with a circularly polarised feed horn driven with 500-W continuous wave from a transmitter. The receiver has a noise figure of 2 dB and a bandpass of 500 cycles and the equipment had a very distinct note because of a spurious frequency near the fundamental; on the Moon-Earth circuit it is very easy to identify this signal because of this unique characteristic. On the day in question a series of dots or a single dash were being reflected back from the Moon after 2.6 s. Suddenly there appeared a second signal delayed by approximately 2 s. This signal had the same characteristics of the Moon Bounce signal except that it was weaker.

At the time of the observations it was afternoon, the Sun was almost due west and the Moon was to the south-west with an altitude of about 30°. Throughout a series of transmissions the returning Moon signal was followed about 2 s later by the delayed ghost signal with the same characteristic note of the transmitter. Unfortunately, I could not record the signals though they continued for 20 min. When I failed to track the Moon with my antenna, the Moon signal would fade but the echo remained at about the same strength.

The following day a severe radio blackout occurred, and lasted for several days, coincidentally with the appearance of a large sunspot. Relating my reception of the ghost echo with this violent solar eruption I suggest that the large streamer of gas from the corona of the Sun produced a highly ionised cloud which reflected the radio signals I had directed towards the Moon. If this streamer approached with a speed of 1,000 km s⁻¹, with a front like a shock wave, it could have acted as a good reflector. The cloud would have been about 800,000 km out in space, as indicated from the delay times of 4-5 s.

HANS LOHMANN RASMUSSEN

Amateur Radio OZ9CR,
 Asum Odense Denmark

Received February 11; accepted July 16, 1975.

²²Na, Ne-E, extinct radioactive anomalies and unsupported ⁴⁰Ar

A NEW picture¹ of the origin of the known extinct radioactivities (¹²⁹I and ²⁴⁴Pu) holds that these radioactive species were precipitated in grains forming in the rapidly cooling ejecta of explosive nucleosynthesis, and that their decay occurred in interstellar grains rather than in the meteorites. If so, our interpretation of extinct radioactivities is enlarged. Their detectability is no longer related to the usual criterion that they live long enough for the meteorites to form, but rather that they live long enough for grains to form in the expanding envelope.

My first point is to interpret ²²Na as a detectable extinct radioactivity. Its half life (2.6 yr) seems long enough for an expanding gas to cool to the point of grain formation. If we take the adiabatic relationship $p/T^3 = \text{constant}$ for purposes of a simple estimate, we find that matter having a density of

10⁴ g cm⁻³ at $T=10^9$ K has a density of 10⁻¹⁴ g cm⁻³ when $T=10^3$ K where grains can form copiously. The observations² of Nova Serpentis 1970 confirm very extensive grain formation in similar circumstances on a time scale of a few days. If the expansion speed of a supernova envelope is 10⁴ km s⁻¹, this drop in density requires about 3 yr. Condensation temperatures may be reached even earlier if the expansion is not adiabatic, but also radiates.

The ejection of ²²Na in large amounts is expected from the helium shells of explosive supernovae³ and from the surface explosions of novae⁴, both at the level 10⁻³ g g⁻¹. I have studied these for their prospects for nuclear gamma-ray astronomy, but since ²²Na may in part be expected to precipitate in grains before it decays, we may also expect the existence of interstellar grains being isotopically rich in ²²Ne in comparison with the Sun. The admixing of these grains without vaporisation into the forming carbonaceous chondrites is a good candidate for the source of the ²²Ne-rich neon component Ne-E, which was discussed by Black⁵, who attributed it to an extrasolar source. It is at least as ²²Ne-rich as ²⁰Ne/²²Ne < 3.4, and could conceivably be pure ²²Ne. The fact that the released gas is purest in Ne-E around 1,000 °C in stepwise heating probably reflects the fact that ²²Ne occupies a refractory ²²Na site in the grains. The absolute amounts of ²²Ne_E are also relatively constant near 10⁻⁹ cm³ g⁻¹ STP, suggesting uniform sprinkling of presolar grains through C1 chondrites.

The ²²Na yield is more than adequate to account for the Ne-E anomaly. Nucleosynthesis calculations suggest, but do not prove, that the fraction of all ²²Ne that was synthesised as ²²Na, in specific ejecta concentrations near 10⁻³ g⁻¹ g (refs 3, 4), is about 10⁻², which is also about 5 × 10⁻² of the total stable ²³Na abundance. This total nucleosynthesis yield is quite large, and about one-tenth of it is here supposed to be incorporated into grains before the ²²Na decay. I have argued¹ that the fraction f_{Na} of condensed solar system Na that existed as unvaporised presolar grains lies between 10⁻¹ and 10⁻². If these estimates are correct, the concentration of Ne-E potentially available if neon gas were not lost from grains is between 5 × 10⁻⁴ and 5 × 10⁻⁵ of stable Na. That this is many orders of magnitude greater than observed Ne-E concentrations reflects the loss of daughter neon from the hot but unvaporised grains.

A correlation of Ne-E with Na concentration would not necessarily be expected, however, because these same explosively ejected zones are not rich in stable ²³Na. This Ne-E is a component of Ne-A (ref. 5), which shares a "threshold-for-appearance" effect with He-A, which is deficient in ³He in comparison with the solar wind. Component A is interpreted by Black⁵ as a linear combination of E and a component D, which he interprets as a primitive solar wind, before the deuterium has burned to ³He. In the framework of the present discussion, we see that D may also contain the dense gas surrounding the newly formed grains in the expanding supernova. I suggest interpreting E as ²²Ne from ²²Na decay and essentially pure ⁴He from the helium shell in which the ²²Na is synthesised³. Other isotopes of neon, especially ²¹Ne, also exist in the gas, but $(^4\text{He}/\text{Ne})_{\text{gas}} \approx 10^4$ so that ⁴He should be the dominant trapped gas. Only if neon is trapped more efficiently than helium in this environment will the newly synthesised neon gas contribute a component to E as well. Calculations of this neon synthesis have been published^{6,7}. Arnould and Beelen⁷ noted the similarity to Ne-E of special cases of the total gas yield, but the ²²Na interpretation seems more plausible since it is much more easily trapped than neon gas.

Black⁵ also calls attention to the fact that in the C2 chondrite Nagoya the dark gas-rich phases contain 3 times as much ⁴⁰Ar as the light gas-poor phases, although the light phases have a higher fraction of Ne-E in their total neon. Perhaps the explanation is that the dark phases contain a higher fraction of presolar grains. The ⁴⁰K/⁴¹K ratio was initially much higher in nucleosynthesis events, so grains forming there rather than in the solar system would now have more ⁴⁰Ar/⁴¹K. This suggestion is not necessarily inconsistent with the bulk ²⁰Ne/²²Ne

ratios, because the dark gas-rich portions may contain absolutely more Ne-E than the light phases. It has simply been diluted by more captured gas. In any case, it must be pointed out that such grains would seem to have "unsupported ^{40}Ar ". Interstellar grains accreted by the lunar surface, as I have suggested¹ from unsupported fission xenon, would also show unsupported ^{40}Ar . Since unsupported ^{40}Ar has been found in lunar soil⁹, this idea as a partial source should not be overlooked.

The views expressed here suggest many correlations to look for. Calcium should be a rich element for anomalies. The greatest may be at ^{44}Ca , which we⁸ have shown is synthesised as 47-yr ^{44}Ti . Titanium is also refractory, so both elements condense out early, but a $^{44}\text{Ca}/^{40}\text{Ca}$ correlation with Ti/Ca is here predicted in the small scale structure. The heaviest isotopes of Ca are, moreover, probably synthesised¹⁰ in different mass shells from ^{40}Ca , so they may also be expected to show small scale anomalies. Since ^{41}K is synthesised as radioactive ^{41}Ca (ref. 8), small ^{41}K excesses correlated with calcium concentration are predicted. Anomalies due to ^{26}Al should also be interpreted in terms of presolar grains rather than a primordial concentration.

Our calculations⁸ show that ^{53}Cr is synthesised as parent ^{53}Fe , which is arrested at 2×10^6 yr ^{53}Mn . In grain formation, therefore, a substantial part of this yield will chemically correlate with Mn. That is, a correlation of $^{53}\text{Cr}/^{52}\text{Cr}$ with Mn/Cr is predicted. Peculiarities at ^{54}Cr are also expected, in as much as it is synthesised in a separate process¹¹.

Another outstanding candidate is iron: ^{54}Fe is synthesised¹¹ along with $^{56,57}\text{Ni}$, which are themselves held up for months to years before their transmutation to $^{56,57}\text{Fe}$ is complete. Grains forming in this period could show Ni or Co correlated anomalies due to chemical effects. They will also be deficient in ^{58}Fe , which is synthesised elsewhere¹¹. Other possibilities suggest themselves once the key idea is in mind.

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DONALD D. CLAYTON

Department of Space Physics and Astronomy,
Rice University, Houston, Texas 77001

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Preferential orientation in four C3 chondritic meteorites

It is more than a century since oriented fabrics were first noted in chondrites (see ref. 1), but since then little work has been done on the topic^{2,3}, the most extensive being that by Dodd⁴. He found that most of his H- and L-group chondrites possessed foliation, together with lineation in the most strongly foliated specimens. The preferential orientation of elongated olivine phenocrysts in porphyritic chondrules has also been observed⁵.

A visual examination of the Leoville C3 chondrite disclosed a strong orientation of the long axes of the chondrules. This prompted us to compare the orientation of the long axis of the chondrites in this meteorite with three other Type 3 carbonaceous chondrules: Grosnaja, Coolidge and Vigarano. We were unable to obtain specimens cut with three mutually perpendicular faces, the most desirable configuration for a study of this kind. Our preliminary samples generally possessed only one face cut

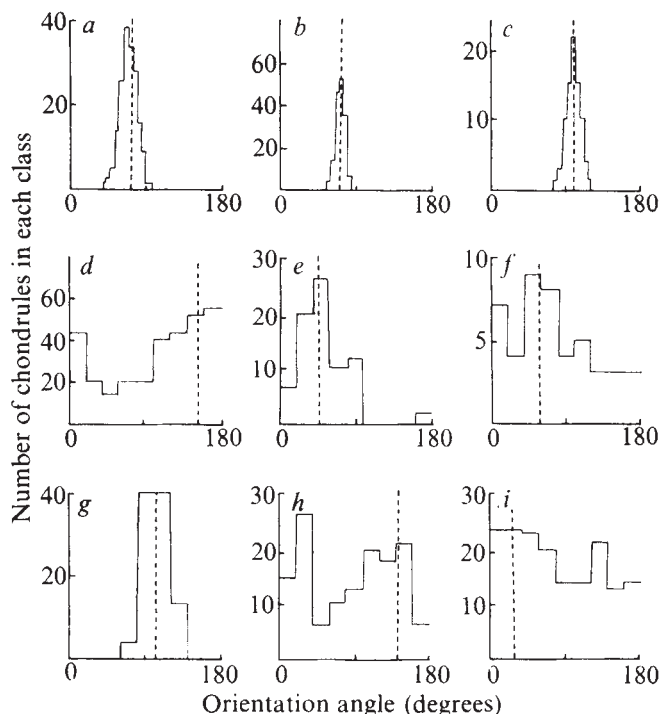


Fig. 1 Histograms of the orientations of the long axes of chondrules in four C3 carbonaceous chondrites. Leoville specimens: a (LU 44484), vector magnitude (V_m) = 94.1%; b (BM 1969, 144), V_m = 98.5%; c (LU 44484), V_m = 95.6%. Three faces of Vigarano specimen (BM 1924, 15); d, V_m = 29.3%; e, V_m = 67.8%; f, V_m = 24.1%. Grosnaja specimen (BM 63624): g, V_m = 86.1%. Two specimens of the Coolidge chondrite (BM 1959, 854): h, V_m = 14.1%; i, V_m = 11.2%. Marked lines, mean vector directions.

at random, and we may, therefore, have encountered some of the problems outlined by Dodd⁴.

We thus decided to photograph each available face using fine-grained monochrome film, and then to prepare appropriate enlargements. This avoided the problem posed by the removal and transport of actual specimens and facilitated magnification to a suitable size. Individual photographs were mounted on the table of an orientometer designed to allow movement in translation and rotation⁶, and were viewed through a binocular microscope fitted with a line reticule in one eyepiece. Chondrules were aligned with their long axis along this fiducial line, and their nominal orientation was read from the degree scale of the orientometer turntable. Each measured image was marked with a spot of ink to avoid confusion and possible duplication. The measurements are of the double-headed vector type⁷, so only the direction between 0° and 180° was taken.

Chondrule orientations for each specimen were grouped into 5°, 10° or 20° classes according to the number of measurements and their scatter, and the resulting histograms are shown in Fig. 1. The mean vector direction and its magnitude are also shown, complete orientation being expressed as 100% and a random distribution by 0% (ref. 7).

Histograms give a good visual idea of the presence and degree of any orientation and our measurements indicate a high degree of orientation of the long axes of chondrules from all specimens of the Leoville chondrite, in the Grosnaja specimen, and on face b of the Vigarano specimen (Fig. 1 a, b, c, e and g). The other two faces of Vigarano (Fig. 1 d and f) exhibit a much lower degree of chondrule orientation, and the Coolidge specimens (Fig. 1 h and i) show a wide scatter. These qualitative impressions are borne out by the calculated vector magnitude.

From these results it seems that the orientations of the long axes of chondrites are strong in Leoville and Grosnaja chondrites, moderate in one plane of the Vigarano specimen, and very weak or nonexistent in the Coolidge specimens. Dodd⁴ could find no preferred orientation in the last-named

meteorite. The wide range of degrees of orientation between the four C3 chondrites examined also supports Dodd's conclusion⁴ that there is no correlation between orientation and degree of metamorphism.

The identification of the mechanisms which produced asphericity and alignment of the chondrules in some chondrites is not an easy matter. Are these phenomena related to the primary chondrule-forming process, to effects occurring during aggregation, or are they a result of secondary processes in the meteorite parent body? Are the two phenomena related? More data are needed before these questions can be resolved.

For this reason we are at present examining the orientations of the long axes of chondrules in more detail, along with such factors as shape and size distribution, density, friability, and so on. It is hoped that these studies will enable us to draw conclusions concerning the formation of chondrules and the genesis of meteorites.

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PHILIP M. MARTIN
A. A. MILLS
ELAINE WALKER

Departments of Astronomy and Geology,
University of Leicester,
Leicester LE1 7RH, UK

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Possible ozone depletions following nuclear explosions

HAMPSON¹ has commented on the possible destruction of the Earth's ozone shield by nitric oxide generated during nuclear explosions in the atmosphere. He suggested that if such an ozone decrease were large enough and lasted long enough, it might have disastrous effects on the biosphere. Here, we assess quantitatively Hampson's suggestion.

We have considered only nuclear detonations occurring near the ground, with the NO formed and carried into the stratosphere by rising fireballs. High altitude bursts would probably be much lower in total yield; moreover, NO injected above the stratosphere has a shorter photochemical lifetime and is less effective in removing ozone than is NO deposited in the stratosphere. To calculate the quantity and distribution of NO in the atmosphere after a series of large nuclear explosions, we have assumed that each megaton of nuclear yield generates 10^{32} molecules of NO, an average value between upper and lower limits^{2,3}. We have assumed that the NO is uniformly mixed in the nuclear cloud at stabilisation, when it has a spheroidal shape and lower and upper altitudes given by the empirical curves of Peterson⁴ for 30° to 90° latitudes.

For the total yield we have chosen two values: 5,000 Mton (case 1), as suggested by Hampson¹, and twice that, 10,000 Mton (case 2), in order to assess a more severe case. In both cases the total yield was provided equally by 1 Mton and 5 Mton devices. This choice is approximately consistent with published tabulations of strategic nuclear armaments⁵ delivered by missile. To determine the sensitivity of the results to the individual yields, an additional case consisting of 5,000 Mton provided equally by 1 Mton and 3 Mton devices, has also been treated. The injected NO is assumed to be uniformly distributed either between 30° and 70°N, or over the entire Northern Hemisphere. The latter

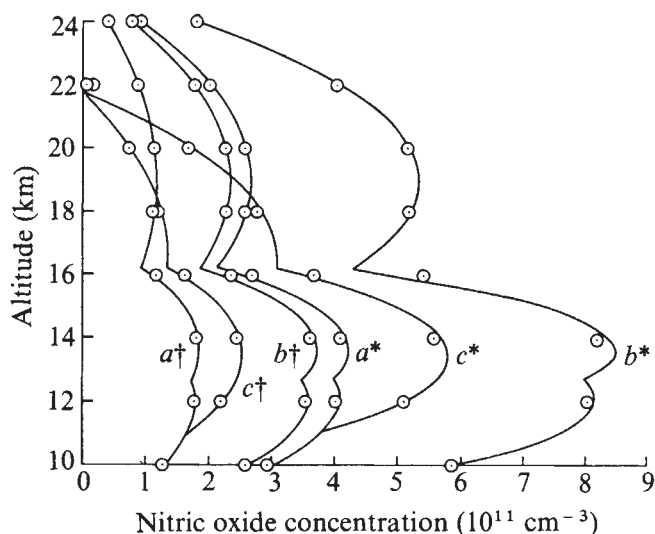


Fig. 1 Initial concentrations of nitric oxide generated by nuclear explosions for total yields of: a, 5,000 Mton produced equally by 1 and 5 Mton devices; b, 10,000 Mton produced equally by 1 and 5 Mton devices; c, 5,000 Mton provided equally by 1 and 3 Mton devices. The NO molecules are assumed to be uniformly spread over: *, zone between 30° and 70°N; †, the Northern Hemisphere. Each circle point represents an average concentration for a 2-km vertical interval (the model grid spacing) centred at that altitude; that is, it is an average concentration in each 2-km segment.

case is included because hemispherical dispersion can occur in a time (several months) which is short compared with the stratospheric residence time of the NO (several years). The 'initial' NO concentrations for each case are shown as functions of altitude in Fig. 1.

If the nuclear bursts actually were confined between 75°–125°W and 25°–75°E, corresponding roughly to the geographical boundaries of the USA and the populated USSR, it would take about as long for the NO to spread uniformly over the area between 30° and 70°N (ref. 6), as it would take for the ozone column density to reach a minimum (about 2 months). Accordingly, our calculations using longitudinally averaged NO concentrations are only estimates of the average ozone reductions to be expected. A higher initial concentration of the NO_x generated by fireballs in certain geographical regions ensures that the ozone reduction will be somewhat larger in those areas.

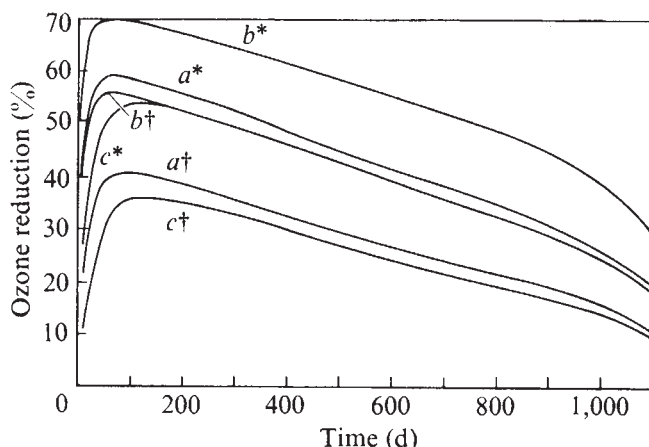


Fig. 2 Depletion of the total ozone column as a function of time after a series of large nuclear explosions for cases described in Fig. 1.

The depletion of ozone and atomic oxygen by catalytic reaction with nitrogen oxides produced by nuclear explosions ($\text{O}_3 + \text{NO} \rightarrow \text{O}_2 + \text{NO}_2$; $\text{O} + \text{NO}_2 \rightarrow \text{O}_2 + \text{NO}$) was assessed using a one-dimensional numerical model of stratospheric and mesospheric constituents that includes 150 photochemical reactions (see ref. 7). The model predicts the concentrations of 46 chemical constituents; only the species O, $\text{O}(\text{D})$, O_3 , OH, HO_2 , NO, NO_2 and HNO_3 need to be considered here. The vertical transport of stratospheric gases is represented by an eddy diffusion process; we have selected a diffusion coefficient⁸ which reproduces the vertical distribution of methane at altitudes above 30 km and also gives the proper stratospheric residence times for radioactive tracers (H. S. Johnston, P. Kattenhorn, and G. Whitten, unpublished). This model, however, has a tropopause level at an altitude of 16 km, which is appropriate only for low latitudes; between 30° and 70° the average tropopause is near 11 km. Accordingly, we have shifted the Wofsy and McElroy⁸ diffusivity curve downward by 5 km. The predicted ozone reductions are, of course, sensitive to the eddy diffusivity profile used. In view of the constraints placed on vertical turbulent transport rates by the tracer data, we believe that uncertainties in the calculated ozone depletions resulting from uncertainties in the eddy diffusion coefficients are much less than a factor of two, and are probably not greater than 25%. Our calculations were performed with the Sun at a fixed elevation of 45°. It is valid⁹ to assume a fixed rather than a diurnally varying solar zenith angle when predicting the effects on ozone of artificially injected NO_x .

Figure 2 shows our computed ozone column depletions as a function of time after a large-scale nuclear detonation for combinations of yields and subsequent horizontal spreading. In cases *a* and *b* the peak ozone reductions occur after about 2 months, with large depletions persisting for several years. When the lower weapon yields of 1 Mton and 3 Mton are assumed (case *c*), the NO is injected lower in the stratosphere (see Fig. 1) and our results show slightly smaller ozone reductions for the same total yield as in case *a*, and about twice as long a delay to the peak ozone reduction. The *e*-folding time for ozone recovery is about 3 yr in our model, reflecting the slow transfer of NO_x compounds from the stratosphere to the troposphere by eddy diffusion. After about a year the NO_x should be spread fairly widely throughout the northern hemisphere⁶, thus reducing its concentration to about half the value it would have had were it still confined to the 30°–70°N zone. Comparison of the *a*[†], *b*[†] and *c*[†] curves in Fig. 2 with the corresponding * curves shows that this spreading decreases the ozone reduction by about 20–30%. In the former cases, however, the ozone reduction occurs over the entire Northern Hemisphere, not just in the zone between 30° and 70°N.

Figure 3 shows the post-detonation variation of the ozone concentrations at several altitudes for case *b** in Fig. 7; that is, NO produced by 10,000 Mton distributed between 30° and 70°N. Below the tropopause (~11 km altitude), excess NO_x is so quickly removed by rainout, and its catalytic reaction cycle with ozone is so slow because of low atomic oxygen concentrations, that there is little ozone reduction. In the stratosphere up to about 22 km, the peak ozone reduction occurs more quickly with increasing height as a consequence of the increasing abundance of O. Most of the NO_x above 22 km has been transported upwards by diffusion from lower altitudes, so the peak ozone reduction occurs later for higher altitudes, reflecting the time required for upward transport of NO by eddy diffusion. Eventually, all of the excess stratospheric NO_x is transported either downward into the troposphere where it can be washed from the air by rainfall or upwards into the mesosphere where it can be photolysed into N and O and destroyed by the subsequent reaction $\text{N} + \text{O} \rightarrow \text{N}_2 + \text{O}$.

Our calculated ozone depletions are only estimates because of the expected inhomogeneous distribution of NO produced

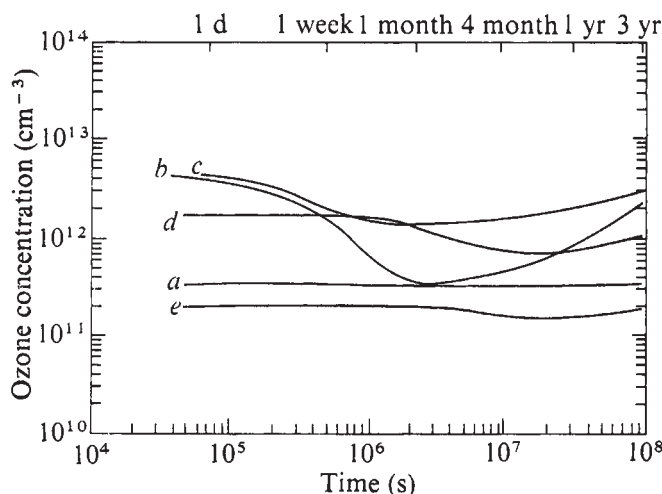


Fig. 3 Variations of the ozone concentrations over time at several altitudes for case *b** in Fig. 1. (10,000 Mton zonally distributed): *a*, 10 km altitude; *b*, 16 km altitude; *c*, 26 km altitude; *d*, 36 km altitude; *e*, 46 km altitude.

by nuclear explosion, because of uncertainties in the total amount of NO generated (perhaps a factor of 2) and its initial altitude distribution, and because of probable substantial changes in stratospheric circulation caused by very large ozone depletions. Moreover, our computations apply to mid-latitude conditions, that is, somewhere between 30° and 70°N. For more accurate calculations, a photochemical model with multidimensional transport including thermally-driven winds is required. We do not, however, expect the results from such a study to differ markedly from those reported here, and we therefore conclude that Hampson's concern is well founded.

R. C. WHITTEN
W. J. BORUCKI

NASA-Ames Research Center,
Moffett Field, California 94035

R. P. TURCO

R & D Associates,
Santa Monica, California 90403

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Trapa natans in the British Flandrian

POLLEN of *Trapa natans* L. the water chestnut, has been found in two Flandrian (Holocene) deposits at Skipsea, North Humberside, providing the first *in situ* Flandrian records of this species in Britain. The occurrence, followed by the extinction, of *T. natans* in Scandinavia has been regarded as evidence of a 'climatic optimum' when summer temperatures were at least 2 °C higher than at the present day¹⁻⁴. The new British data may indicate similar conditions but could also record human activity, in which case it would not be necessary to invoke climatic change.

We studied two former meres occupying depressions on the surface of the Devensian (Weichsel) till. The pollen was

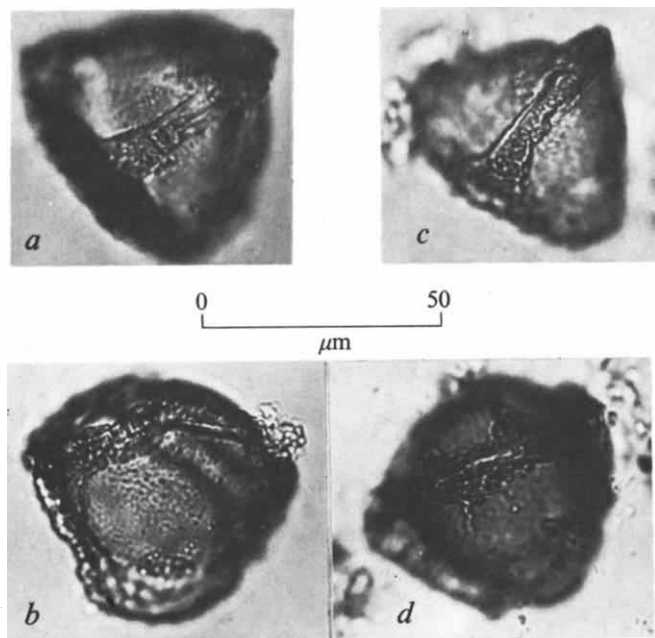


Fig. 1 Pollen grains of *Trapa natans* L. a, b, Reference pollen; c, fossil grain from 2.75 m, Skipsea Bail Mere; d, fossil grain from 2.87 m, Skipsea Low Mere.

found in the fine detritus mud of a 5.50-m core from the site of the former Skipsea Low Mere⁵ (os ref.: TA 155563) at Skipsea, near Hornsea, North Humberside. The total number of *Trapa* grains seen in pollen preparations of samples taken at 0.25 m intervals is shown in Table 1.

The pollen was also present in fine detritus mud of a 4.00-m core from the site of Skipsea Bail Mere⁵ (os ref.: TA 162552) nearby. Examination of prepared slides revealed single grains at 2.75 m, 2.90 m, and 3.25 m, but not in intermediate samples.

The pollen was determined as *Trapa* by comparison first with drawings⁶ and photographs⁷, and, subsequently, with reference material (Fig. 1). *T. natans* is the only species of Trapaceae found at present in western Europe.

There are at least four possible explanations of the find: laboratory contamination, derivation from interglacial deposits, introduction by birds, and actual occurrence of the

Table 1 Total number of *Trapa* grains seen in complete scanning of pollen preparations 0.25 m apart in a core from Skipsea Low Mere

Depth	Number of grains
2.12 m and above	0
2.37 m	1
2.62 m	2
2.87 m	12
3.12 m	1
3.37 m and below	0

species at the two meres in the Flandrian. Contamination seems very unlikely, since no specimen or sample of *T. natans* or of any deposit known to contain it had ever entered our laboratory previously. It is certainly possible that the grains are derived (indeed one specimen is somewhat eroded); for that to be correct, however, requires that the grains were derived twice: first from an interglacial deposit into the Devensian till which surrounds the meres, and then into the mere deposits. The samples contained no other pollen of types which are necessarily derived, nor did they have the appreciable content of mineral matter which could be expected if derivation from till had occurred. Transport of pollen by birds is possible. Seeds and fruits adhere to birds⁸, and pollen may travel in the same way. The nearby Hornsea Mere is an important staging post for waders migrating from Scandinavia (J. E. S. Walker, personal communication) at a time when *T. natans* is flowering (June–September³). Carriage by birds cannot, therefore, be excluded, but should be considered as a remote possibility in view of the regular appearance of the pollen at several stratigraphical levels. The presence of *Trapa* pollen probably indicates that the taxon was living at the site⁹.

The single previous record from the British Flandrian¹⁰ is of a fossil fruit found floating in a brackish loch in the Outer Hebrides. Doubt has been cast on this record⁴ because of the general unlikelihood that the species would occur in so oceanic an environment: in addition the fruit had peat (apparently of the *Sphagnum*–*Calluna* type) adhering to it, suggesting a bog habitat, whereas *T. natans* is a floating aquatic. Those objections do not, however, apply to the records reported here: North Humberside is not particularly oceanic and the pollen was found in fine detritus mud, representing an environment in which floating aquatics could have existed. The new records should be accepted, provisionally at least.

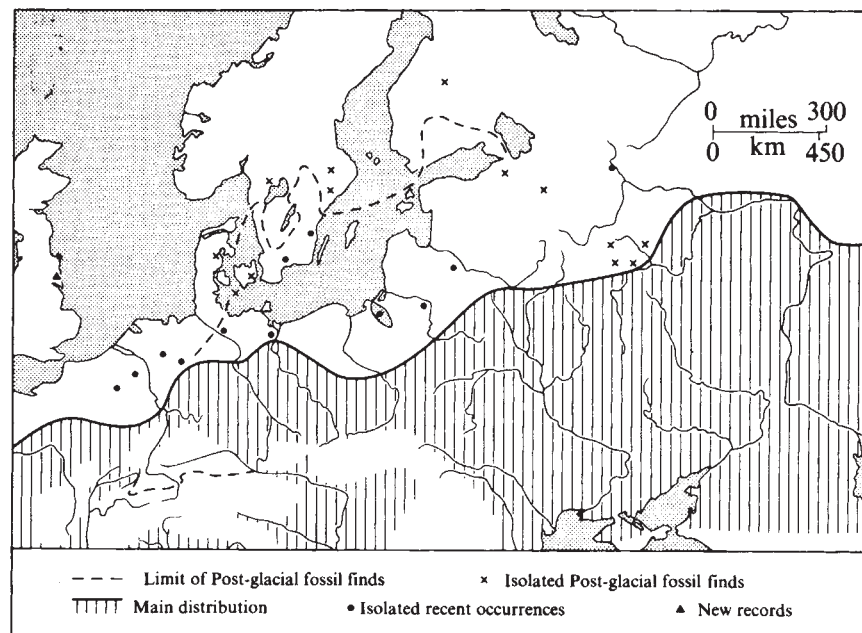


Fig. 2 Distribution of *Trapa natans* (partly after ref. 3).

A pollen and spore count from the main *Trapa* horizon, 2.87 m deep at Skipsea Low Mere, gave the results shown in Table 2.

Outline pollen diagrams showed that the other samples containing *Trapa* from the two meres give similar spectra. These results suggested that *T. natans* was growing in North Humberside after the decline of *Ulmus* but before the main lowland forest clearances (that is, about 2500–500 bc, the period of Bronze Age cultures in Britain). That is the time during which *T. natans* attained its maximum northward extension in Europe, reaching Finland and southern Sweden (Fig. 2)^{1,2}. The arrival of *Trapa* in Northern Europe is usually attributed to ordinary, if rather slow, migration in response to the warm, postglacial climate, although cultivation by man has also been suggested.

Table 2 Pollen and spore count from the main *Trapa* horizon, 2.87 m deep at Skipsea Low Mere

<i>Pinus</i>	1	<i>Corylus</i>	60	<i>Sparganium</i>	2
<i>Ulmus</i>	5	Gramineae	3	<i>Trapa</i>	1
<i>Quercus</i>	68	Cyperaceae	1	Polypodiaceae	3
<i>Tilia</i>	10	Caryophyllaceae	1	Filicales	1
<i>Alnus</i>	85	<i>Solanum</i>	1	Deteriorated grains	4
<i>Fraxinus</i>	8	<i>Nuphar</i>	1		
Total pollen and spores 255					

Suggested causes of the decline of *Trapa* include³ climatic deterioration, a decline in the trophic status of fresh waters, the extinction of beavers, the collection for food by man, the cessation of cultivation as a food plant, disease, and degeneration caused by inbreeding. Most authors^{1–4} prefer a climatic explanation and correlate the decline with the aftermath of the 'climatic optimum' when summers were at least 2 °C warmer than at present. The new records could be used to support this figure.

Explanations relating to man, however, deserve re-examination in the context of the new records. Water chestnuts have been an important source of food in Europe and elsewhere⁵. It is to be expected that a species on the edge of its range will be unusually sensitive to the depredations of man. The activity of a late Bronze Age culture in the area is confirmed by the presence of a substantial lake settlement in Skipsea Low Mere and another in the marshy area between Skipsea Low Mere and Skipsea Bail Mere¹. It seems likely that people living there would have taken advantage of this readily available food supply, and may thereby have caused its extinction. Alternatively, man could have introduced and cultivated water chestnuts. That would explain their arrival, and their extinction may have coincided with the decline of late Bronze Age culture.

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J. R. FLENLEY
B. K. MALONEY
D. FORD
G. HALLAM

Geography Department,
University of Hull,
Hull HU6 7RX, UK

Received June 9; accepted July 14, 1975.

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A new method of measuring the hydrogen bond stretching frequency ν_o of a complex in solution

THERE is no agreed explanation of the broadening of the infrared absorption bands associated with the $\nu_o(\text{XH})$ stretching mode of a hydrogen-bonded complex $\text{XH}\cdots\text{Y}$ in solution (see ref. 1). There is, however, a growing body of evidence to suggest that the distinctive appearance of these bands can be understood in terms of the anharmonic coupling of $\nu_s(\text{XH})$ and $\nu_o(\text{XH}\cdots\text{Y})$ modes^{2,3}, provided it is recognised that the phase coherence of the $\nu_o(\text{XH}\cdots\text{Y})$ mode is rapidly lost owing to the strong interaction between the complex and the solvent⁴. The implication of this proviso is that the $\nu_o(\text{XH}\cdots\text{Y})$ motion should be regarded as an oscillatory stochastic process rather than the oscillation of a conservative system.

A model developed by one of us (G.N.R., unpublished), which assumes an explicit form for the anharmonic coupling between the $\nu_s(\text{XH})$ and $\nu_o(\text{XH}\cdots\text{Y})$ modes and invokes the Ornstein–Uhlenbeck stochastic process to represent the hydrogen bond stretching vibration, allows a simple expression for the molecular dipole moment autocorrelation function to be obtained. This theoretical autocorrelation function depends on three parameters: (1) a mode–mode coupling constant; (2) a viscous damping coefficient, representing the effect of the solvent on the $\nu_o(\text{XH}\cdots\text{Y})$ mode, and (3) the angular frequency of the hydrogen bond stretching vibration. By Fourier transformation of digitally recorded infrared spectra we have obtained experimental dipole moment autocorrelation functions for the phenol–acetonitrile complex in solution in carbon tetrachloride and for a number of other systems. Using the method of least squares we have been able to estimate numerical values and standard deviations for the three parameters of the model in each case. In particular, we have been able to measure the hydrogen bond stretching frequency with an accuracy of about 10%.

We believe that this provides the first absolutely conclusive evidence that the $\nu_s(\text{XH})$ band profiles (Fig. 1) of hydrogen-bonded species in solution are indeed composite. Furthermore, our method of analysis provides a means of measuring the $\nu_o(\text{XH}\cdots\text{Y})$ frequency in cases where the structure in the 3,000 cm^{-1} region of the spectrum is impossible to resolve visually and the far infrared spectrum cannot be recorded.

The theoretical result which is fundamental to the analysis is obtained by requiring that the displacement coordinate r_2 of the $\nu_o(\text{XH}\cdots\text{Y})$ mode should obey the Langevin equation

$$\ddot{r}_2 + \beta\dot{r}_2 + \omega_2^2 r_2 = F(t) \quad (1)$$

If the random force $F(t)$ acting on the complex is supposed to be a Gaussian random variable with an infinitesimal correlation time, $r_2(t)$ is a representation of the Ornstein–Uhlenbeck stochastic process, the mathematical properties of which are well understood⁵. By assuming a coupling term in the molecular hamiltonian of the form $K_{112} r_1^2 r_2$ and treating the $\nu_s(\text{XH})$ vibration as a quantum mechanical oscillator coupled to a classical stochastic oscillator, it is easy to obtain the dipole moment autocorrelation function

$$\Phi(t) = \exp \left\{ -i\omega_1 t - a^2 \langle r_2^2 \rangle \right. \\ \left. \times \int_0^t (t-x) e^{-\beta x/2} \left[\cos \tilde{\omega}_2 x + \frac{\beta}{2\tilde{\omega}_2} \sin \tilde{\omega}_2 x \right] dx \right\} \quad (2)$$

where $\hbar\omega_1$ is the $\nu_s(\text{XH})$ energy quantum, $a = K_{112}/m_1\omega_1$, and $\tilde{\omega}_2 = (\omega_2^2 - \beta^2/4)^{1/2}$ in the underdamped case. A similar, but aperiodic, result holds in the overdamped case where $\beta > 2\omega_2$. It is worth noticing that β is determined⁵ by the variance of the

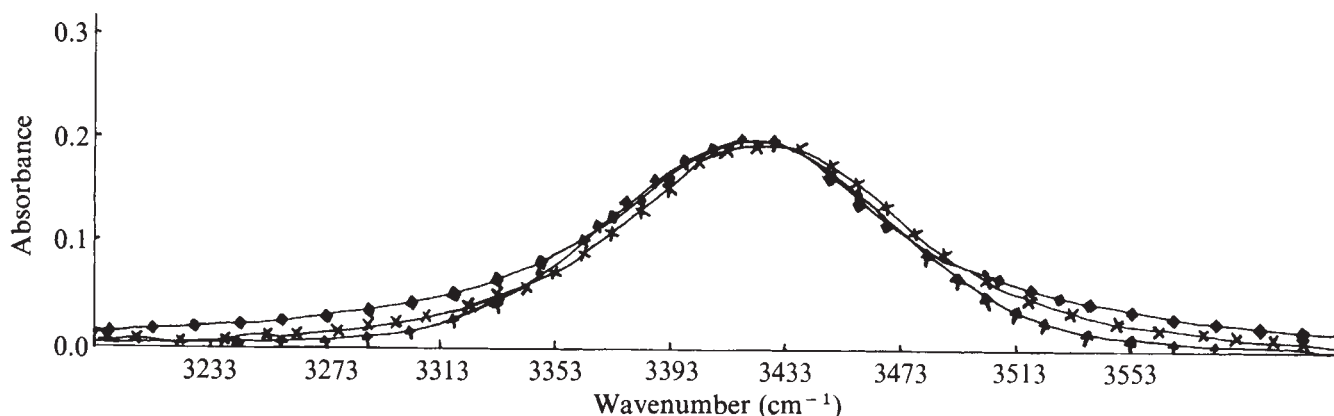


Fig. 1 Comparison of observed ν_s band profile ($\times$) with those of pure Gaussian ($\dagger$) and pure Lorentzian ($\blacklozenge$) bands for phenol-acetonitrile in carbon tetrachloride.

random force $F(t)$. A complete derivation of equation (2), indicating the limits of its applicability, is being prepared for publication elsewhere.

For small t this correlation function behaves as

$$|\varphi(t)| = \exp(-\frac{1}{2}\Delta^2 t^2) \quad (3)$$

and for large t it has the asymptotic form

$$|\varphi(t)| \sim C \exp(-\Delta^2 \tau_c t) \quad (4)$$

where the amplitude of modulation Δ and the correlation time τ_c of the $\nu_s(\text{XH}\cdots\text{Y})$ vibration are given by

$$\Delta = a\langle r_2^{-2} \rangle^{1/2} \quad (5)$$

and φ

$$\tau_c = \beta/\omega_2^2 \quad (6)$$

According to Kubo's general theory of frequency modulation by a Gaussian random process⁶, the former expression for $|\varphi(t)|$ is a good approximation for all times if $\tau_c \Delta \gg 1$, the latter if $\tau_c \Delta \ll 1$. In these limiting cases of slow and rapid modulation the spectral profiles are Gaussian and Lorentzian respectively. Published theoretical work on the band shapes of hydrogen-bonded species in solution has either assumed the extreme slow modulation limit⁴ or has attempted to interpolate between the two limiting cases⁷.

The experimental autocorrelation function for the phenol-acetonitrile complex (Fig. 2) shows clearly that neither limiting case is strictly applicable. Since the $\nu_s(\text{XH})$ band in monomeric phenol is close to Lorentzian in form, it seems likely that quite different mechanisms are responsible for the band shapes of the monomer and the complex. This provides indirect support for the view that the coupling of internal modes of the complex influences its spectrum, which is the basic assumption of the present model. Local field effects may well be of some importance⁸, but are not considered here.

Fitting the experimental autocorrelation function to expression (2) above, using the nonlinear least-squares method of Marquardt⁹ as modified by Fletcher and Powell^{10,11}, we obtain the following estimates of the parameters for phenol-acetonitrile in solution in carbon tetrachloride at 22.5 °C:

$$\Delta = (14.8 \pm 0.2) \times 10^{12} \text{ s}^{-1}$$

$$\Delta^2 \tau_c = (11.9 \pm 0.4) \times 10^{12} \text{ s}^{-1}$$

$$\text{and } \bar{\nu}_2 = 120 \pm 12 \text{ cm}^{-1}$$

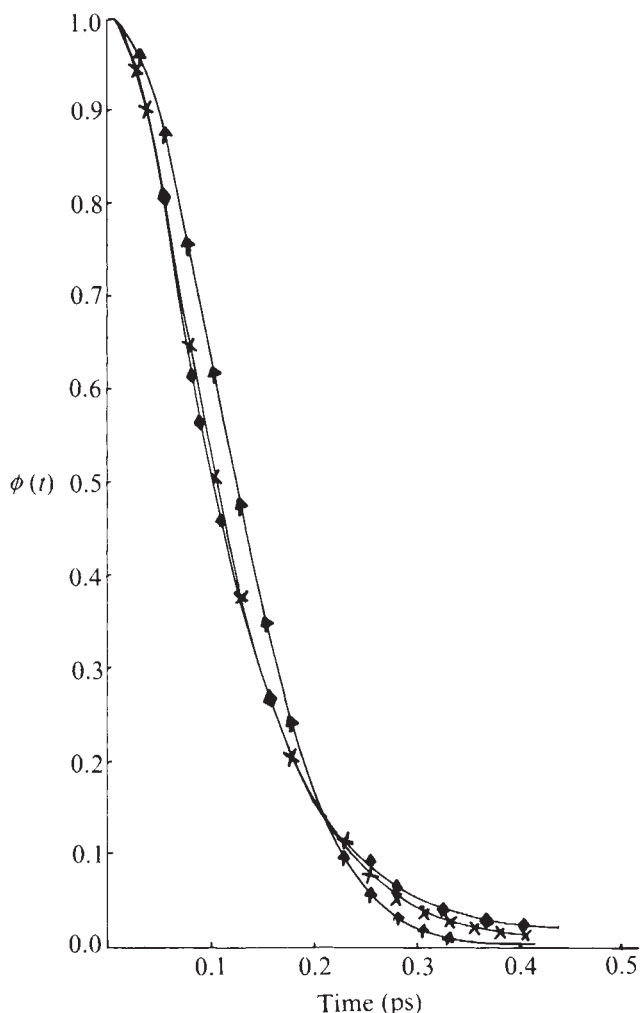
$$\text{where } \bar{\nu}_2 = \omega_2/2\pi c$$

It follows that $\tau_c \Delta = 0.8$ and $\beta/2\omega_2 = 0.6$. We observe that $\tau_c \Delta$ is close to unity, which confirms that neither the slow nor the rapid modulation limiting approximation is satisfactory.

Although the $\nu_s(\text{XH}\cdots\text{Y})$ motion is underdamped, it is not far from being critically damped, and this suggests that the far infrared spectrum of the complex in solution should be extremely broad. We have failed so far to observe the far infrared spectrum.

It should be particularly noted that we are able to calculate the standard deviation of our measurement of the hydrogen bond stretching frequency. Although we cannot exclude the

Fig. 2 Comparison of observed dipole correlation functions obtained by transformation of the spectral bands shown in Fig. 1.



possibility of systematic errors (arising from the crudity of the theoretical result on which the analysis is based, or even from bias in our sampling procedure) we must point out that no other method of measuring the $\nu_s(\text{XH}\cdots\text{Y})$ frequency allows a reliable estimate of the errors of observation to be made. We believe therefore that our procedure must be regarded—by default—as the most trustworthy method yet devised for measuring the hydrogen bond stretching frequency of a complex in solution. It must be admitted, however, that the model makes no provision for any spectral broadening produced by local field effects or by the involvement of rotational and bending degrees of freedom, and that these questions need urgent attention.

We have not attempted in this preliminary report to discuss the temperature dependence of the position of the $\nu_s(\text{XH})$ band centre, although it is relatively straightforward to do so by introducing a further coupling term in the model hamiltonian.

There are several ways in which the model may be tested more stringently. First, the modulation amplitude parameter Δ of equation (5) should decrease by a factor of nearly $2^{-1/2}$ on deuteration. This we have verified for the phenol-dioxan system. Secondly, changing the solvent or varying the amount of excess base (which we have found to produce distinct spectral changes, thus confirming previous work on similar systems^{12,13}) should affect the viscous damping parameter β and thus affect the correlation time τ_c of equation (6).

Finally, changes in temperature should affect both Δ and β . The former, being proportional to the root mean square amplitude of a quantum mechanical harmonic oscillator, should vary as $[\coth(\hbar\omega_s/2kT)]^{1/2}$, or as $T^{1/2}$ at sufficiently high temperatures. The latter, being a viscosity coefficient, is expected to decrease exponentially with increasing temperature. Further experimental work on these problems is in progress and will be reported at a later date, together with a more detailed analysis of experimental errors.

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J. YARWOOD

Department of Chemistry,
University of Durham, Durham DH1 3LE, UK

G. N. ROBERTSON

Department of Physics,
University of Cape Town,
Rondebosch 7700, South Africa

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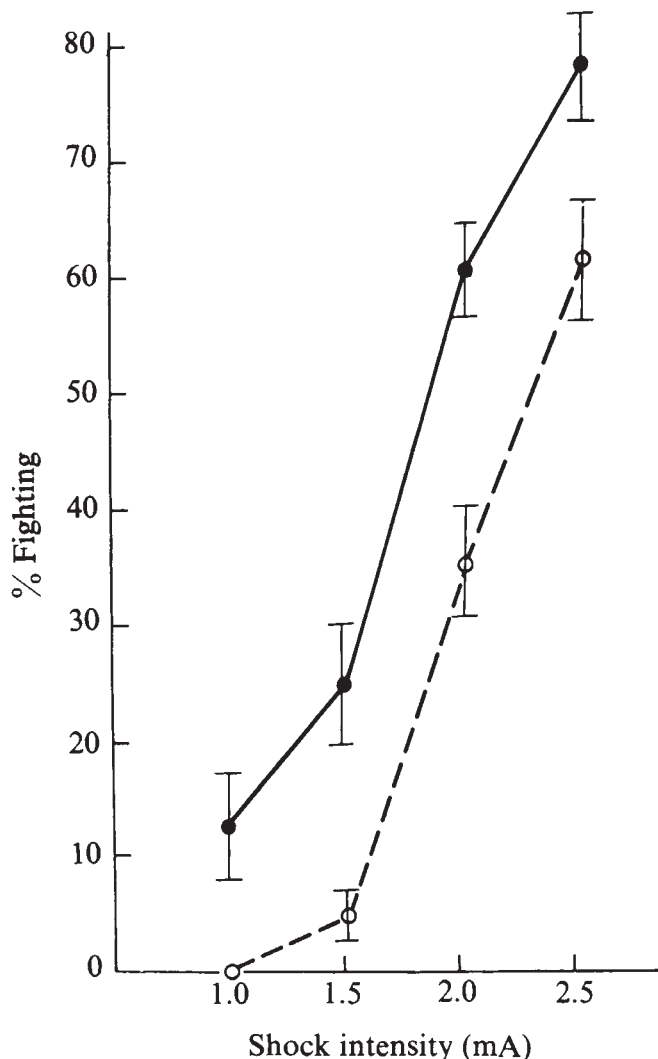


Fig. 1 Mean percentage fighting $\pm$ s.e.m. at each of four shock intensities, 10 shocks each at 1, 1.5, 2 and 2.5 mA when the background noise was either 75 dB (solid) or 55 dB (dotted) for eight pairs of rats. Duration of shock 1 s, 15 s between shocks, 2 min between each shock intensity. Sprague-Dawley male rats 300–350 g, $30 \times 28 \times 24$ cm Plexiglas test cage in sound-attenuated chamber. White noise intensity measured by General Radio Model 1551-C sound level meter. The pairs of rats placed in test chamber were not previously housed together. Half the pairs received 75 dB first, half 55 dB. Three days later pairs retested in reverse conditions.

the present experiments was shock-elicited aggression in the rat. Two rats were paired in a small enclosure and subjected to a series of footshocks which elicit fighting, depending on the intensity, frequency and duration of electric shock^{1,2}. This is a well documented and highly reliable form of aggressive behaviour that is usually considered a form of irritable aggression³.

The results of Experiment 1 are shown in Fig. 1, which plots mean percentage fighting [(observed fights/total possible fights) $\times$ 100] at each of four shock intensities when the background noise level was either 75 or 55 dB. The number of fights increased as the intensity of shock increased but at each shock intensity the number of fights was greater in the 75 dB compared with the 55 dB condition (F (shock intensity) = 60.9; d.f. = 3/21; $P < 0.001$; F (noise) = 23.46; d.f. = 1/7; $P < 0.001$; F (interaction) = 1.22; d.f. = 3/21; $P > 0.10$). This experiment indicates therefore that footshock-elicited aggression was increased by raising the level of background noise.

It is possible that noise increases shock-elicited aggression by increasing arousal. Many arousal mechanisms exhibit non-monotonic relationships to stimulus intensity where the behavioural effect is greatest at intermediate levels of stimulus

Effect of noise on shock-elicited aggression in rats

ONLY a limited number of experiments have been designed to evaluate the effects of noise on aggression. As animal models of aggression are amenable to pharmacological and physiological analysis we have investigated the effect of noise on aggression in rats and have found an interesting non-monotonic relationship, with an increase in aggression at moderate noise levels but a decrease at high levels. The aggressive behaviour chosen for

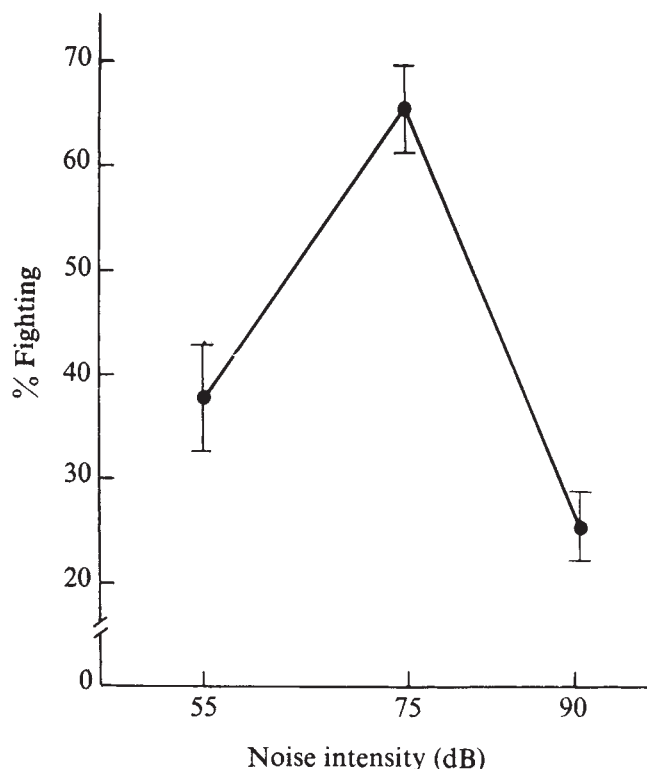
intensity, but less at low or high levels of stimulus intensity. For example, the relationship between the startle response and background noise is a non-monotonic one^{4,5}. Both the startle response and shock-elicited aggression may be considered to reflect types of irritability which might be influenced by shifts in arousal. As background noise may be an effective means of manipulating arousal, it was of interest to determine whether shock-elicited aggression would also bear a non-monotonic relationship to the level of background noise. Experiment 2 was designed to evaluate this possibility.

Figure 2 shows the mean percent of fights across the 30 shocks at each of three levels of background noise and indicates that the relationship between this measure of aggression and noise level was a non-monotonic one ($F(\text{quad}) = 110.37$; d.f. = 1/16; $P < 0.001$). Thus, there was an increase in aggression when the noise level was raised from 55 to 75 dB ($t = 3.40$; d.f. = 8; $P < 0.001$), but a decrease in aggression when noise level was raised to 90 dB ($t = 5.21$; d.f. = 8; $P < 0.001$). In addition, there was even less fighting at the high intensity of background noise than at the low intensity ($t = 2.11$; d.f. = 8; $P < 0.05$).

Experiment 3 was designed to evaluate whether the length of exposure to noise before testing would influence subsequent aggression. Different groups ($n = 9$) were exposed to either 55 or 75 dB noise for either 1, 5 or 15 min. After pre-exposure, a series of 30, 2 mA shocks were presented at the same noise level used during pre-exposure. The results indicated that although there were highly significant increases in the number of fights during 75 compared with 55 dB noise conditions ($P < 0.001$ in each group) there was no significant effect of varying the length of pre-exposure to noise before testing.

Experiment 4 was designed to evaluate how long the increase in aggression that occurred when testing was carried out at the 75 dB noise level would continue, once that noise was turned down. Nine pairs of rats were each given a series of 40, 2-mA shocks at a 15-s intershock interval 5 min after being placed in the test cage. For three pairs the background noise was 75 dB throughout, for three other pairs it was 55 dB throughout. For

Fig. 2 Mean percentage fighting $\pm$ s.e.m. across 30 shocks at 55, 75 and 90 dB background noise. Nine pairs of rats. Design: 3×3 latin square, testing at each noise level followed every other level equally often. 30, 2 mA shocks given at 15 s intervals. The 55 dB point represents baseline noise level with the noise generator turned completely off.



the last three pairs the noise was 75 dB for the first 5 min but 55 dB thereafter. The various pairs were then tested in the other two conditions 3 and 6 d later using a 3×3 latin square to determine the order of testing.

The following results were obtained. When rats were exposed to 75 dB throughout the test period the amount of fighting increased to a maximum level in 2.5 min and remained at this level thereafter. Rats exposed to the 75 dB noise level which was then reduced to 55 dB also quickly increased their frequency of fighting to a peak at 2.5 min. Thereafter the frequency of fighting rapidly declined, so that after approximately 3.75 min it was equal to that of rats exposed to 55 dB throughout. The last two experiments indicate therefore that the effect of noise on shock-elicited aggression is most dependent on the level of noise present during testing where the effect develops and decays very rapidly.

The present series of experiments demonstrate that noise can influence shock-elicited aggressive behaviour. The precise mechanism which mediates this effect remains to be investigated. One possibility might be that noise increases arousal. Increasing arousal by electrical stimulation of the reticular formation⁶ or by amphetamine⁷ has been shown to facilitate aggressive behaviour in the cat. Moreover, the relationship between arousal and behaviour often seems to follow an inverted U function⁸ where increases in arousal at first increase the frequency and/or intensity of behaviour but further increases in arousal actually decrease the same behaviour.

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MICHAEL H. SHEARD
DAVID I. ASTRACHAN
MICHAEL DAVIS

Department of Psychiatry,
Yale University Medical School,
and Connecticut Mental Health Center,
34 Park Street,
New Haven, Connecticut 06508

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Strain differences in maze-learning ability of *Drosophila melanogaster*

If it could be demonstrated that *Drosophila* are able to learn, then our extensive knowledge of the genetics of this dipteran could be applied to understanding the biological bases of learning. Reports of learning in *Drosophila*¹⁻³ sometimes involve changes in orientation as the result of aversive stimulation, for example, electric shock. The use of these stimuli can bring additional problems such as conditioned inhibition where the animals cease responding altogether⁴. The present paper is the first report of work on a unique learning situation which is based on the well-known mazes used to study taxes in *Drosophila*⁵ and involves no aversive stimulation.

We have used modifications of 11-unit mazes (Fig. 1), where flies are allowed 24 h to travel from the starting tube to the collection tubes, making 10 right-left choices at junctions connected by one-way cones. Gravitational and extraneous light cues were eliminated by mounting each maze horizontally, with an opaque base, sides and top. The internal parts of the maze were of clear Perspex which allowed the flies to be attracted through the maze by phototaxis, the light source being a 10 W fluorescent lamp, the width of the maze, shining

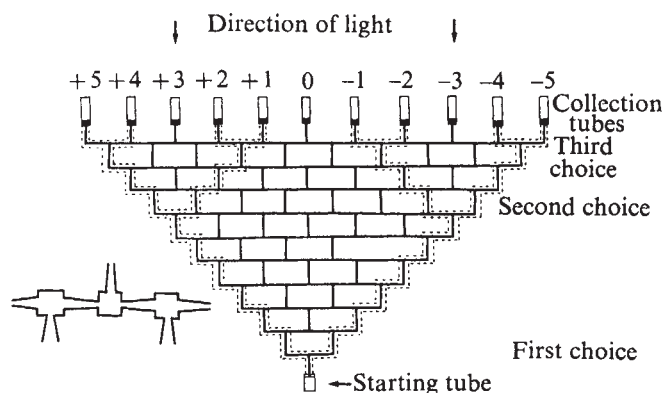


Fig. 1 Schematic drawing of the maze. Dashed lines represent the possible pathways in the modified maze, where the other alternatives are blocked off. Dimensions: 62 × 49 cm. Small drawing shows a typical junction with the interconnecting one-way cones.

from behind the collection tubes. In such a maze, one does not know the sequence of turns made by the flies. If the number of choices is reduced from 10 to 3, however, by blocking off certain junctions of the maze (Fig. 1), the flies are limited to eight alternative pathways, corresponding to the eight collection tubes, tubes +3, 0 and -3 no longer being used.

This modified maze was used to study the sequence of choices in five replicates of each of 10 strains of *D. melanogaster*, LM20–29, derived from single inseminated females captured 9 yr previously at Leslie Manor in Victoria Australia and inbred mainly by single-pair matings since that time. Genetic variation for other traits, such as mating speed, chaetae number and desiccation resistance exists between these strains⁶. Because locomotor activity changes in young flies⁷, all testing was carried out 3 d after eclosion: 100 males and 100 females of the same strain were put in the starting tube (diameter 2 cm, depth 1 cm), providing sufficient crowding to induce the flies to enter the maze. Compared with other experiments where only 50 (ref. 2) or 60% (ref. 8) of the flies completed the task in the allotted time, an average $92.1 \pm 7.8\%$ of these 200 flies reached the collection tubes (10 × 2.5 cm culture vials) at the other end of the maze.

Unlike conventional maze studies where subjects are run through the same maze several times, the flies here were only

tested once. The index of learning involved comparing the probabilities of going left or right at the start of the maze and the probabilities of repeating this response at the second choice-point indicated in Fig. 1, termed PL, PR, PLL and PRR, respectively. Between these two choices the flies were forced to make six turns, to the left in the case of those initially turning left and to the right for those initially going right. If the behaviour of the flies is unaffected by the six forced turns, then the values of PLL and PRR should be similar to those of PL and PR, any change being denoted by $CL = PLL - PL$ and $CR = PRR - PR$. Significant values of CL and CR indicate that when presented with a choice, flies are more likely to continue the sequence of turns they were forced to adopt for the previous six turns. That is, they learn that this sequence of turns enables them to proceed through the maze towards the light, which presumably also acts as an orientational cue in their turning.

All the necessary statistics can be calculated from the distribution of flies in the eight collection tubes. PL is the proportion out of all the flies in these eight tubes that are in the four tubes on the left side and that therefore must initially have turned left. PLL is calculated as the proportion out of the flies from the four left-side tubes that are in the two furthest left tubes (+5 and +4). PR and PRR are calculated similarly for those flies initially turning right.

Analyses of variance (Table 1) revealed significant differences between the strains in PL, the initial tendency to go left. Moreover, as found in earlier work⁸, flies are initially more likely to turn left than right (overall mean PL = 0.5446). Such a turning bias has been observed in other arthropods⁹ and cannot simply be attributed here to asymmetry of the maze, as any such influences would be the same for each strain (the testing of the five trials of each strain were randomised between three identical mazes, each completely separated by matt-white partitions from the other mazes)—that is, asymmetry alone does not explain the observed differences in PL among the strains.

From the significant strain differences in CL and CR (Table 1), we see that after six forced turns to the left or right, the flies of some strains are more likely to choose to continue turning this way. The overall mean values for CL and CR of 0.1644 and 0.2655 respectively indicate that the increase in this probability is considerably less for left- than for right-turning, although the latter was initially the less frequent choice.

Flies that learn follow the outer walls of the maze, and it is necessary to consider alternative factors to learning that could lead to flies behaving in this way. One crucial experiment demonstrates that such alternatives could not contribute to the strain differences in learning.

Table 1 Values of PL, CL and CR for the ten inbred strains, and analyses of variance

Strain	PL	CL	CR
LM20	0.5592 ± 0.0458	0.2888 ± 0.0696†	0.3729 ± 0.0776†
LM21	0.5737 ± 0.0703	0.1970 ± 0.1153	0.3231 ± 0.0900*
LM22	0.5491 ± 0.0606	0.0945 ± 0.0874	0.1701 ± 0.1033
LM23	0.5191 ± 0.0452	0.1586 ± 0.0943	0.2631 ± 0.0801*
LM24	0.5015 ± 0.0545	0.3273 ± 0.0781†	0.3009 ± 0.0948*
LM25	0.5666 ± 0.0646	0.1139 ± 0.1403	0.3139 ± 0.0768†
LM26	0.6053 ± 0.0493	0.0318 ± 0.0707	0.3254 ± 0.1066*
LM27	0.4500 ± 0.0574	0.2261 ± 0.0588*	0.1511 ± 0.0814
LM28	0.5407 ± 0.0654	0.1435 ± 0.1382	0.1458 ± 0.0794
LM29	0.5810 ± 0.0467	0.0623 ± 0.0748	0.2881 ± 0.0969*
Item	d.f.	Mean square	Mean square
Strain	9	3.61*	9.20†
Sex	1	0.061	3.89†
Strain			
× sex	9	0.92	0.39
Between replicates	80	1.60	0.65

PL is the initial probability of turning left, CL the change in this probability after an initial left choice and six forced turns and CR the corresponding change in the probability of going right after an initial right turn. Means s.e. of five runs of 200 flies (100 of each sex). The values of CL and CR which differ significantly from zero are indicated thus (d.f. = 4 in all cases). * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

Mean squares for analyses of variance have been multiplied by 100. The between-replicates variance could be used as an error variance only for PL, as the corresponding variances for CL and CR differed significantly between the strains, those with low means having larger variances, presumably because their choices were more random. Transformations of the data were attempted but did not reduce heterogeneity of the variances.

Five sets of 200 flies (100 of each sex) of strains LM20, 26, 27 and 28—which vary from both CL and CR being significant to neither being significant (Table 1)—were tested in the normal 11-unit maze with no passages blocked off and where flies thus were not forced to repeat a sequence of turns. Otherwise the mazes were identical. The mean percentages $\pm$ s.e. of flies ending up in one or another of the outermost tubes (that is, +5, +4, -4, -5) were, for each of the above strains, 35.6 ± 3.9 , 42.8 ± 4.7 , 21.4 ± 1.9 and 30.8 ± 1.5 , respectively. Although these results do show that some strains are more likely than others to follow the outside wall and so end up in the end tubes, these strain differences bear no relationship to the differences in CL and CR in the eight-unit maze—that is, large values of CL and CR in strains like LM20 cannot simply be the result of flies following the maze walls without learning.

That other strains like LM28 follow the walls to the same extent as LM20 in this maze but show no significant CL or CR in the eight-unit maze eliminates explanations of the learning by factors such as a light gradient, where flies merely follow the outer walls because of cues provided by light getting through the black Perspex baffles at each side of the maze or shining from the maze end, but being reflected differentially from the outer and inner maze surfaces. Although they are already excluded by this 11-unit maze experiment, there is some more specific evidence to eliminate other possible alternatives to learning for the eight-unit maze performance:

Stampede effect, where flies follow each other in groups³. At 2 and 4 h after starting the 200 flies in each maze, the means $\pm$ s.d. of the number of flies having reached the collection tubes were 49.5 ± 34.1 and 89.8 ± 28.7 , respectively. On both occasions the distribution of flies matched the final distribution after 24 h. This would not have been the case if following were of major importance as the distribution should then show clumps of flies in particular collection tubes.

Wall-hugging (following the outer walls of the maze) and its converse, **centrifugal swing** affect maze performance in other invertebrates¹⁰. These factors may be relevant in other *Drosophila* maze studies^{8,11} but are less likely here, where the junctions have many small corners with both curved and flat surfaces to be negotiated (Fig. 1, detail). Moreover, observations of flies in transparent-topped mazes show that they can explore a junction for as long as 45 min before proceeding, rather than going straight through the maze, as a wall-hugging and centrifugal swing hypothesis would require.

Right-left bias. At the initial choice point, flies are being separated by their preference for turning left or right and it is simply those strains with a greater initial bias in one direction or another that are more likely to repeat the choice at the second choice-point. Turning seems to be probabilistic, however, and there is no evidence that some individual flies consistently turn left and others right. All the flies reaching the +5 tube during the five trials of each strain were stored on fresh food and rerun through the maze (-5 flies were similarly treated). If the bias explanation were correct, then the +5 and -5 flies would be those with a strong bias to turning left or right respectively at choice-points in the maze. Presumably this bias should be indicated on the rerun by increases in the initial probability of going left (PL) for the +5 flies, and right (PR) for the -5 flies, but in none of the 10 strains were the values of PL and PR significantly different from the original values in Table 1. Similarly, 27 generations of separate selection (from an F_2 population) of flies going to +5 or -5 has produced some increase in CL and CR, but not the change in PL and PR that a bias explanation would predict (D.A.H., unpublished). Also, if a right-left bias were important, the probabilities of repeating the same response at the third and final choice-point should be higher than the earlier probabilities. The opposite is the case, although possibly this third choice is affected by the flies being close to the end of the maze and being able to smell the food medium in the collection tubes (see Fig. 1) and so need not imply that learning has been lost by this stage of the maze.

Sequential alternation. When an organism is forced to turn one way in a maze and then given a choice, it will sometimes turn the opposite way, demonstrating what is termed sequential alternation⁴ or correcting behaviour¹². Although this may be considered a form of learning—the animal must retain some memory of the way it was forced to turn—and could be important in other mazes where passages are not blocked off and where *Drosophila* do repeat turns or sequences of turns⁸⁻¹¹, it cannot be the sole explanation here, for the same reasons that wall-hugging and centrifugal swing have been excluded.

Additional possibilities such as the olfactory cues postulated in other maze studies¹³ have been tested and excluded. The remaining explanation is that the flies display associative learning. With no apparent positive or negative reinforcement, they associate passage through the maze towards the light with a sequence of turns or, more accurately, with a particular orientation towards the light at each choice-point, since, as mentioned previously, flies may move about the junction for a considerable time before actually making the choice. Thus the behaviour here can be considered a form of the exploratory learning which is well-documented in the feeding and homing

of other arthropods¹⁴. Putnam⁹ has explained similar behaviour in the beetle *Aleochara bilineata* as evidence of learning—the beetles make the first choice in a Y maze at random, but the probability of repeating this choice increases on successive trials even without extrinsic reinforcement. He ruled out alternatives like right-left bias and odour cues discussed here, and concluded the beetles learn they can proceed through the maze by turning in this direction. As they never try turning the opposite way at a choice point, they do not find that this alternative response would be equally effective. Both there and in the present mazes, mere progress seems to constitute some differential reinforcement for repeating the same choice. Putnam also refers to work on rodent learning in mazes with reinforcement in both arms that can be explained in the same way.

Thus, genetic differences in learning have been demonstrated in *Drosophila*. The learning task is unusual but allows large numbers of flies to be scored, as it only requires the introduction of flies into the start tube and scoring 24 h later of the final distribution in the collection tubes. As there is no other interference by the experimenter, it should prove easier to replicate these results than has been the case with other *Drosophila* learning situations¹⁵. Like the aversive conditioning techniques¹⁻³ learning is being considered here at the population, rather than the individual level. But there has already been considerable success in the genetic analysis of taxes in mazes similar to these⁶, and the present technique may offer an effective screen for localising chromosomal factors determining the learning ability of *Drosophila*.

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D. A. HAY

Department of Genetics and Human Variation,
La Trobe University, Bundoora,
Victoria 3083, Australia

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Two photosystems controlling behavioural responses of *Halobacterium halobium*

Halobacterium halobium contains a retinal-protein complex, bacteriorhodopsin, which is the only protein of the so-called purple membrane forming distinct patches within the surface membrane^{1,2}. Bacteriorhodopsin acts as a photoreceptor molecule and is chemically similar to the visual pigment rhodopsin^{1,3}. On illumination bacteriorhodopsin undergoes a fast cyclic photoreaction with at least four intermediates occurring after microseconds and milliseconds⁴⁻⁶. Various experiments indicate that bacteriorhodopsin functions as a light-driven proton pump which builds up a proton gradient across the cell membrane and is used for ATP synthesis^{7,8}. Thus its primary function, different from that of rhodopsin in the eye, seems to be energy transformation⁷. Moreover, *H. halobium* shows light dependent motor responses, and so we supposed that bacteriorhodopsin also has a sensory function. To test this possibility we obtained action spectra for the light-induced behavioural responses from *H. halobium*. We

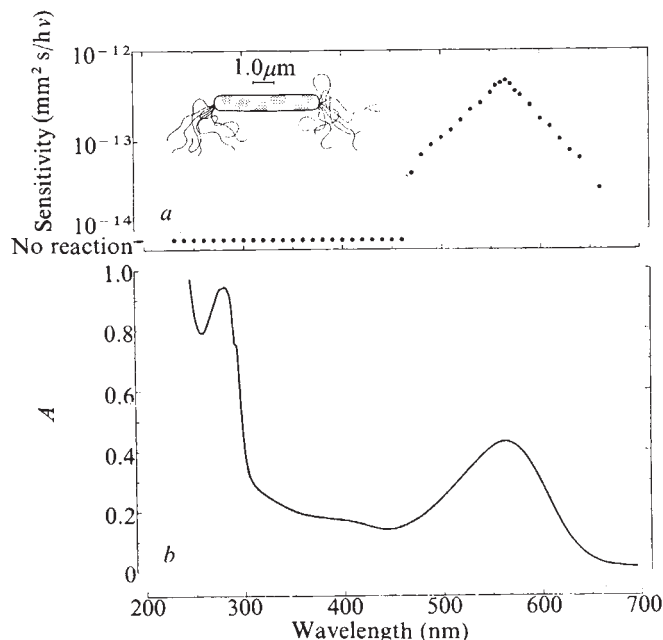


Fig. 1 *a*, Action spectrum of the inverse photophobic response (IPR) of *H. halobium* occurring after decrease of light intensity. Background illumination ($\lambda = 420\text{--}490 \text{ nm}$) was given through the condenser of a phase contrast microscope. Stimulating light (half width $\leq 10 \text{ nm}$) generated by a xenon source (XBO 150 W) or a mercury lamp (HBO 150 W) in connection with a high intensity grating monochromator, Bausch and Lomb, visible range, or a Zeiss monochromator type M4 QIII, respectively, was applied through a quartz objective connected with an incident light illuminator. Light intensity was measured by means of a light detector UDT 40 connected with pin 10 or pin 10 ultraviolet calibrated against a thermopile. Ordinate: sensitivity in log units given as the reciprocal of the change in light intensity which evokes the response after 1.9–2.0 s. Standard deviation of the means does not exceed the diameter of the points ($n = 4$). Room temperature. *b*, Absorption spectrum of the isolated purple membrane (bacteriorhodopsin) in H_2O after purification on a linear sucrose density gradient.

found two photosystems, one of which shows that bacteriorhodopsin is involved in the light-controlled motor response.

Experiments were performed with the mutant strain R_1 of *H. halobium*, lacking gas vacuoles. The bacteria were usually in the stationary growth phase, during which a maximum of purple membrane is present. These rod-shaped bacteria are about 4–10 μm long and 0.5–0.7 μm in diameter. Tufts of flagella on both poles, visible in electron micrographs of negatively stained bacteria, enable the cell to swim in both directions of its long axis. The mean swimming rate was 2.3 $\mu\text{m s}^{-1}$ (at 24 °C). Sudden changes in light intensity elicit a phobic response. After a latent period of about 1 s the bacterium stops moving for about 1 s and after axial rotation starts swimming in roughly the opposite direction. Similar responses can be evoked either by an increase of light intensity, called the direct photophobic response (DPR), or by a decrease of light intensity, the inverse photophobic response (IPR). In both cases the time elapsed between the beginning of stimulus and the first visible response depends on intensity, duration and wavelength of the stimulus.

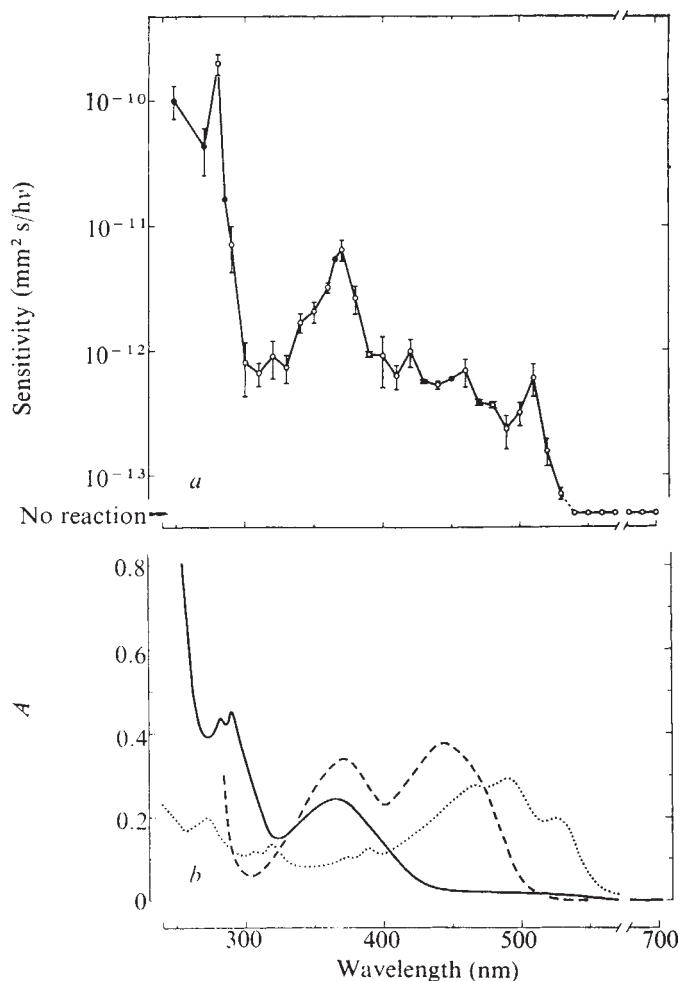
Action spectra of the DPR and the IPR were obtained by measuring the stimulus intensity at the threshold. Single bacteria in a suspension were observed microscopically and the stimulus intensity at different wavelengths was varied until 19 out of 20 specimens examined showed the stop reaction within the time interval from 1.9 to 2.0 s after the onset of stimulus. Stimuli were always maintained until completion of the phobic response.

The action spectrum of the IPR (Fig. 1a) shows a single maximum of sensitivity at 565 nm which corresponds to the peak of absorbance of bacteriorhodopsin in the visible (Fig. 1b).

At wavelengths shorter than 460 nm no IPR could be elicited. We call this photosystem PS 565 and assume that its effective pigment is bacteriorhodopsin. This interpretation is supported by the fact that bacteria from young cultures which have not yet synthesised the purple membrane did not show the IPR. The absence of a 280-nm band in the action spectrum can be explained by (1) the probably insufficient intensity in the ultraviolet of the light source and (2) the limited energy transfer from the protein part to the excitable site of the pigment molecule, as found in frog rhodopsin¹⁰.

In the conditions described above a decrease of light intensity of 720 nW mm^{-2} , that is 2×10^{12} quanta $\text{mm}^{-2} \text{s}^{-1}$, at 565 nm was required to elicit the IPR. From the dependence of the threshold on the latent period the absolute threshold intensity was calculated to be 2×10^{11} quanta $\text{mm}^{-2} \text{s}^{-1}$, corresponding to an estimated 6×10^5 quanta per bacterium per s or 2 quanta per molecule bacteriorhodopsin per s. This relatively low sensitivity compared with that of animal photoreceptor cells indicates that amplification is absent or much less developed. Neither intensity nor wavelength of background illumination affected the height of the threshold. Thus adaptation defined

Fig. 2 *a*, Action spectrum of the direct photophobic response (DPR) of *H. halobium* occurring after increase of light intensity. Background illumination $\lambda > 645 \text{ nm}$; half width of stimulating light 0.5–5.0 nm. Ordinate: Sensitivity as in Fig. 1a. Mean values of 3–11 measurements ($\circ$) or single values and means of two ($\bullet$). Vertical bars represent the standard deviation of the mean. Room temperature. *b*, Absorption spectra of a retinylidene protein obtained after treatment of the purple membrane with 10 mM CTAB (cetyltrimethyl-ammoniumbromide) at $\text{pH} \geq 8.5$ (—), of riboflavin in H_2O (---) and of methanol/ether extraction of pigments from *H. halobium* (mainly α -bacterioruberine) after separation of the purple membrane (....).



as change in sensitivity due to background illumination is absent in PS 565.

The action spectrum of the DPR (Fig. 2a) has two distinct maxima at 280 and 370 nm and smaller secondary peaks towards longer wavelengths. Beyond 530 nm no response could be evoked. With regard to the major peaks we believe a chromoprotein to be the effective pigment of this photosystem, which we call PS 370. The action spectrum resembles the absorption spectrum of a retinylidene protein which can be obtained by CTAB treatment of the purple membrane at alkaline pH (ref. 1) (Fig. 2b). The small peaks in the action spectrum beyond 400 nm may be due to the large amount of carotenoids, mainly α -bacterioruberine¹¹ (Fig. 2b) which could participate in the photoreception or act as a shielding pigment. If the latter is true, the photoreceptor pigment could be a flavoprotein (Fig. 2b), which we consider the more likely because of the marked decrease in sensitivity close to 500 nm. A flavoprotein has also been proposed as a photopigment for other bacteria¹².

To elicit the DPR within a latent period of 2 s a stimulus intensity of 80 ± 10 (s.d.) nW mm⁻², that is $(1.5 \pm 0.2) \times 10^{11}$ quanta mm⁻² s⁻¹ ($n = 7$) at 370 nm and of 3.5 ± 0.8 nW mm⁻², that is $(5.0 \pm 1.2) \times 10^9$ quanta mm⁻² s⁻¹ ($n = 11$) at 280 nm was required. The absolute threshold at 370 nm was calculated to be 1.5×10^{10} quanta mm⁻² s⁻¹. With blue background illumination ($\lambda = 420$ –490 nm) the threshold was 50 times that with red background illumination ($\lambda > 645$ nm) of equal intensity (4 mW mm⁻²). PS 370 developed about 10 h earlier than PS 565 and reached maximal sensitivity immediately whereas the latter was maximally sensitive after 40–50 h. When stimulus intensity and duration were varied the product of both (Bunsen–Roscoe rule) at threshold was constant in PS 370, but not in PS 565.

Our experiments demonstrate that *H. halobium* has two photosystems, as also suggested by H. Berg (personal communication). Both trigger flagellar responses and can be considered as sensory mechanisms. A sensory function implies that the stimulus controls a process which is provided with energy from other sources, that is that the important process is not transduction of energy but of information. Signal amplification, however, as in photoreceptor cells of animals seems not to be essential. Thus, besides the energy-converting function, bacteriorhodopsin acts as a signal transducer.

At present we have no clear idea how sensory transduction may function in *H. halobium*. In PS 565 the excitation could be triggered directly either by light-induced changes in the proton gradient or by an electrical potential across the cell membrane, which might be generated by the electrogenic proton pumping function of bacteriorhodopsin^{13–15}. Control of the motor response through a decrease of stored cellular energy, however, seems unlikely because, among other reasons, of the relatively short latent period even with green background illumination of high intensity, which should keep the cellular energy at a constantly high level. Nevertheless, the response could be triggered by one step of the energy-converting mechanism. If the photopigment of PS 370 is a flavin, a change in electron transport must be taken into account, as has been discussed for the light-induced tumbling effect in *Salmonella typhimurium*¹⁶. By analogy with other cells exhibiting sensory properties, such as receptor cells and protozoa, it should be remembered that changes in the permeability of the cell membrane for certain ions could be mediated by the photosystems and that resulting passive ion fluxes lead to changes in flagellar activity.

The biological relevance of the sensory function is obvious for PS 565. The bacteria do not respond when entering an area of visible light but always reverse their direction if they cross to the dark. Thus they will be trapped in the light which can be used for ATP synthesis. By means of PS 370 the bacteria could avoid ultraviolet radiation which would damage the organisms, as has been shown with mutants lacking carotenoids¹⁶.

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EILO HILDEBRAND

NORBERT DENCHER

Institut für Neurobiologie der Kernforschungsanlage,
D-517 Jülich, Postfach 1913, FRG

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Correlation of electrical and mechanical responses in nervous control of cilia

ALTHOUGH cilia have many important functions in the majority of animal phyla, our knowledge about the mechanism by which their activity is controlled has been fragmentary¹. Extensive electrophysiological studies of the ciliated cell, with intracellular microelectrodes, have been made only with the relatively large protozoans, principally *Paramecium*, where the ciliary activity has been shown to be closely associated with the electrical properties of the cell membrane^{2,3}. Among the metazoans, some examples of nervous control of ciliary activity have been reported⁴, but, mainly because of technical difficulties, intracellular recording of the membrane potential from the ciliated cells has been made in only a few cases^{5,6}; in no case has nervous control of cilia been studied by recording simultaneously the electrical and the mechanical events. Such recording is essential to understanding the mechanism of nervous control at the cellular level.

Using the nerve–cilia preparation made from the gill of the bivalve mollusc, *Mytilus edulis*, we have been able to obtain simultaneous records of neurally evoked mechanical and electrical responses of the ciliated cell. The 'lateral' cilia in this preparation have been known to show an abrupt inhibition of motion, the ciliary arrest response⁷, in response to nerve stimulation, as well as an increased beat frequency which usually follows prolonged repetitive stimulation of the nerve⁸.

A nerve–cilia preparation, consisting of the visceral ganglion and a few gill filaments linked together by a branchial nerve, was dissected out as described previously⁷. From this a nerve-supplied single gill filament preparation was obtained by removing all gill filaments except one. The gill filament was then held, with one of its lateral surfaces facing downward, on the underside of a coverslip placed over a specially designed chamber. A glass capillary electrode with a vertically bent tip, filled with 1.5 M potassium citrate and having a resistance higher than 140 MΩ, was used to penetrate the lateral cell from below. Electrical stimuli were given to the nerve at a distance of 17–20 mm from the site of microelectrode penetration. The mechanical activity of cilia was monitored by projecting their microscopic image through a pinhole on to a photomultiplier tube. The output from the photomultiplier and the membrane potential of the lateral cell were simultaneously recorded on magnetic tape or photographed directly from the oscilloscope screen. The distance between the sites

of recording of the electrical and the mechanical events was 10–20 μm .

With successful penetration, a more or less steady resting membrane potential of about 60 mV (inside negative) was obtained; penetrations which gave potentials less than 30 mV were discarded as these were often followed by a slow depolarisation to zero level. There was no change in the membrane potential corresponding to the cyclic beating of cilia.

When the nerve was stimulated with a 2-ms pulse, there was a transient depolarisation of the lateral cell membrane which took place after a latency of about 120 ms, accompanied by a quick arrest response of the lateral cilia as shown in Fig. 1. The electrical response could be recorded only from the ciliated lateral cells and not from any adjacent regions of the epithelium. The electrical response lasted some 200 ms, of which the depolarising phase (time to peak) was about 30 ms. The amplitude of the response was not constant, but was usually less than 20 mV (mean of 26 measurements, 12.5 mV, 22–25 °C). No overshoot was recorded throughout the experiments. There was a marked facilitation of the electrical response when the nerve was stimulated at about 5 Hz, and also a summation evident at 20–40 Hz.

Repetitive stimulation elicited a prolonged arrest response of the lateral cilia, even when the individual electrical responses were separated (Fig. 2). The mechanical response usually outlasted the repeated electrical responses. On the other hand, the onset of the electrical response preceded that of the mechanical response by about 30 ms. The mechanical response was observed when the electrical response was above about 2 mV, although the threshold depolarisation, as well as the latency, of the mechanical response could not be determined accurately with the present monitoring method.

These results indicate that the transient depolarisation of the lateral cell induced by nervous activity is correlated with the quick arrest response of the cilia, and not with the frequency increase nor with the initiation of each beat. We have previously shown that the response can be induced by a high concentration of potassium⁹, and also by an 'outward' electric current passed across the ciliated membrane¹⁰. The present results are in full accord with these and support the view that the animal can control the cilia by depolarising the cell membrane⁹. They also support the work of Mackie *et al.*⁶ who recorded electrical activity associated with the ciliary arrest response in the ascidian *Corella*.

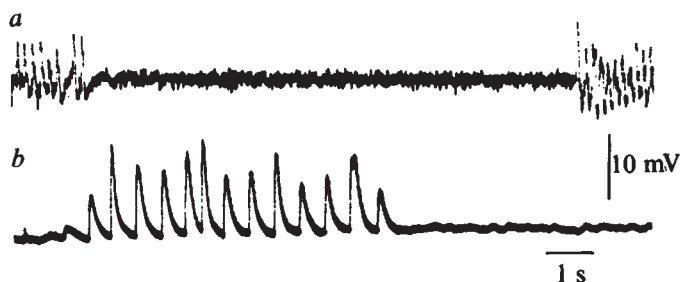


Fig. 2 Response to repetitive stimulation of the branchial nerve at about 2 Hz. *a*, Ciliary activity; *b*, membrane potential. Note that the ciliary arrest response outlasts the electrical activity. Resting membrane potential: –67 mV (26 °C).

In *Paramecium*, calcium has been known to play an essential role in the coupling between membrane depolarisation and the 'reversal' response of cilia³. In *Mytilus*, too, the arrest response seems to depend on calcium since it disappears in calcium-free media¹¹ and also since, in the 'model' cilia made by treatment with Triton X-100, a similar response can be induced by adding calcium ions to the medium¹². The mechanism underlying the arrest response seems to be fundamentally similar to that underlying the ciliary reversal response in Protozoa, as has already been pointed out^{6,9,13}. The ciliary reversal response in certain metazoans is also correlated with the electrical activity of the membrane^{14,15}.

To conclude, the present results support the hypothesis that the ciliary arrest response, as well as the reversal response, is controlled by a mechanism comparable with that which regulates the muscular contraction through membrane excitation and the action of calcium ions^{3,13}.

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A. MURAKAMI
K. TAKAHASHI

Zoological Institute,
Faculty of Science,
University of Tokyo,
Hongo, Tokyo 113, Japan

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Concentration of mercury by three species of fish from Japanese rivers

THE concentration of mercury in aquatic organisms is quite high relative to that in the surroundings but the exact mechanism by which this effect occurs is not known. Generally, higher mercury levels in the environment are reflected in the amount found in protoplasm. Mercury levels in rivers and lakes have increased markedly as a result of industrial discharges; thus, there is a proportionality between mercury concentrations in fishes and in waters, it is possible, using permissible mercury concentrations in fish tissue as a guide (Japan 0.4, US 0.5 p.p.m.), to

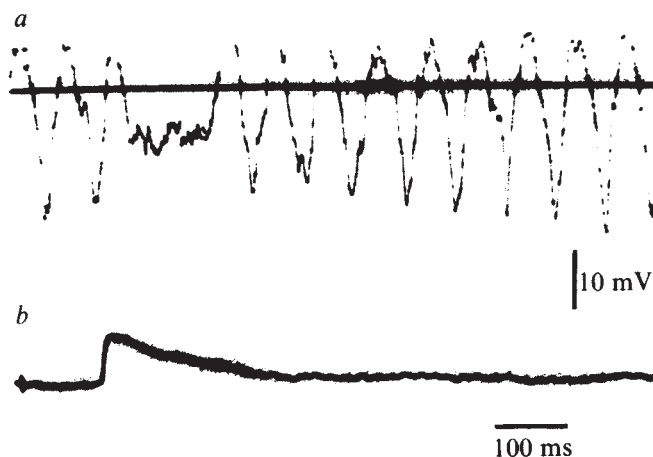


Fig. 1 Ciliary arrest response in the gill of *Mytilus*, elicited by stimulating the branchial nerve with 2-ms pulse given at the beginning of the record. *a*, Photoelectrically recorded activity of lateral cilia. Prevailing oscillatory pattern represents normal beating activity, momentarily suppressed after stimulation. *b*, Intracellular recording of the membrane potential. Note that the onset of electrical response (depolarisation) precedes the mechanical response. Resting membrane potential: –60 mV (21 °C). A reference line indicating –10 mV is superimposed on *a*.

make recommendations for acceptable mercury levels in lakes and rivers. I have found no reports regarding this relationship.

Mercury contents of two rivers receiving waste discharged from mercury mines of an unpolluted river, and of three species of fish (crucian carp, *Carassius carassius langsdorfii*; dace, *Triblodonhakonensis*; zacco temmincku, (Temminck et Schlegel)) feeding mainly on organic matter in water, were determined. Rivers studied were the Hoono in Nara Prefecture, and the

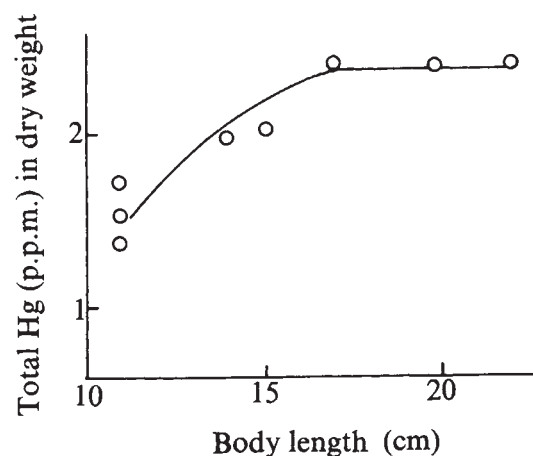


Fig. 2 Mercury concentrations in crucian carp from Hoono River.

Muka and Kunebetsu in Hokkaido. No factories were situated in the immediate vicinity of the sampling area.

Figure 1 shows the relationship between mercury content in three species studied and the mercury level of the river from which they were taken. Figure 2 shows the change in mercury content of one of the fishes as a function of body length. The equilibrium concentration for mercury was found to be 2.4 p.p.m. dry weight or 0.72 p.p.m. wet weight of tissue. The latter suggests a concentration factor relative to Hoono River water of $25,000 \pm 2,100$. On the other hand, methyl mercuric mercury concentrations found in crucian carp were $60 \pm 12\%$ of total mercury, indicating a concentration factor for inorganic mercury of $10,000 \pm 2,600$. From these factors, one may suggest maximum permissible mercury levels for rivers containing these fish based on the permissible wet tissue level; waters should therefore not contain total mercury levels greater than 0.016 or 0.04 parts per 10^9 for inorganic mercury if they are to be fished for those fish having a concentration factor similar to that of crucian carp.

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K. MATSUNAGA

Department of Chemistry,
Faculty of Fisheries,
Hokkaido University, Hakodate, Japan

Received March 21; accepted July 9, 1975.

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Combined effect of ozone and sulphur dioxide on human pulmonary function

THERE have been few studies of the effects of common air pollutants on man during light exercise, and even fewer of the effects of combinations of pollutants. We have found that low concentrations of ozone and sulphur dioxide together have much greater effect on pulmonary function than when either is breathed separately. Reduction of particulate pollution in the ambient air of cities with a high traffic density creates favourable conditions for ozone formation (0.15 p.p.m. ozone has been recorded in London, England¹) and subsequent interaction with ambient SO_2 . In environments where oxidants are already a major problem, air pollution will be compounded by energy-saving measures which encourage the use of heavy (sulphur-containing) fuels. Thus, either increased levels of photochemical pollutants in an atmosphere with already high SO_2 levels or

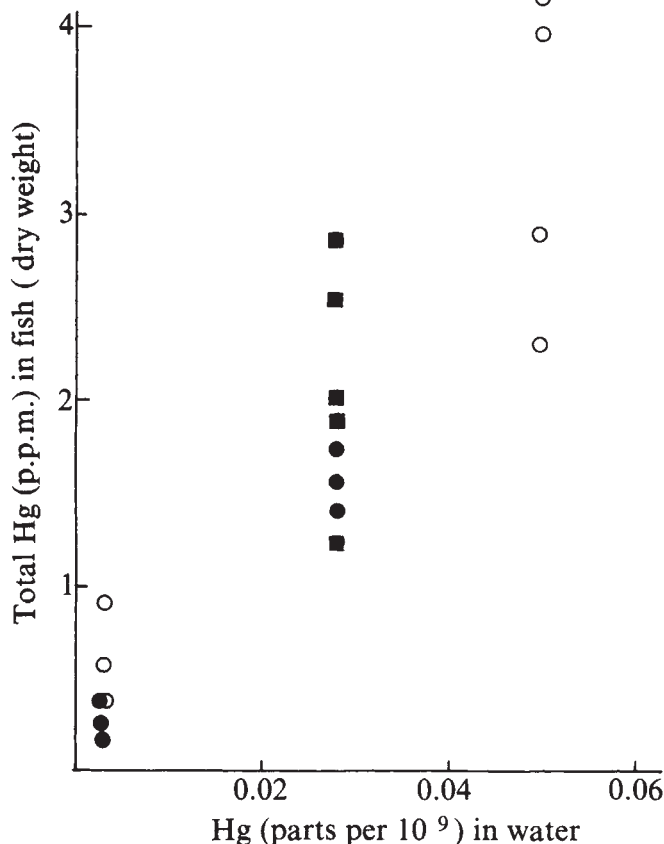


Fig. 1 Relationship between mercury concentration in fish and in surrounding water. $\circ$, Dace (17 ± 1 cm); $\bullet$, crucian carp (11 ± 1 cm); $\blacksquare$, zacco temmincku (17 ± 1 cm). Mean mercury concentrations (parts per 10^9) in river waters. Kunebetsu: $0.004(1) \pm 0.000(8)$, (five determinations); Hoono: 0.029 (two determinations); Muka: 0.050 (two determinations). During the period of study mercury levels remained relatively constant and are therefore considered representative of these rivers. Although polyethylene bottles have often been used for sample storage, I have found that nanogram amounts of mercury are given off by them during storage (about 1 month), which increases the initial mercury level by a factor of 2–100 times, even if the container has been acid-rinsed. When samples were stored in all-glass containers, made 0.4 N with concentrated sulphuric acid and adjusted to 35% with sodium chloride heated to 500°C to drive off mercury, no contamination or reduction of mercury levels was observed. Samples were stored for about 1 month before analysis. The outline for analytical method for water samples is as follows: ionic mercury in a 0.5–0.8 l sample is reduced with stannous chloride and the mercury vapour generated is transferred to gold particles, packed in a glass tube, by a stream of nitrogen gas. The metal is then heated to 500°C in a furnace, and vaporised mercury determined by an atomic absorption spectrophotometer. The error of a single determination is about 10% at the 5 ng absolute level of mercury. Muscles of fish caught in the rivers were immediately removed and dried at room temperature. Analysis of the sample was as follows²: 0.05–0.2 g sample was burnt by passing oxygen gas over it in a quartz tube. The combustion gases were absorbed in a potassium permanganate in 1 N sulphuric acid solution. The mercury in this solution was then determined as with water samples. The error of a single determination was about 7% at the 50 ng absolute level of mercury.

increases in SO_2 concentrations in the presence of oxidants may result in a potential health hazard.

Subjects were eight 19–25-yr-old normal, non-smoking male university students with no history of significant chest illness or atopic illness. They were exposed to SO_2 and O_3 in a chamber that has been described before². Ozone was generated by electrical discharge in oxygen, and SO_2 (partially diluted with air) was added to the chamber from a cylinder. SO_2 and O_3 were added to the inlet of the chamber, and both gases were sampled about 2 feet from the nose of the subjects. Since O_3 will interfere with the analysis of SO_2 and *vice versa* we used scrubbers connected to the intake tubing of the analysers to remove the interfering gas.

SO_2 was removed from the intake, sample tube to the O_3 meter (Mast model 724-2) by a scrubber of glass fibre paper impregnated with chromium trioxide in 60–70% sulphuric acid. O_3 was removed from the sampling tube to the SO_2 meter (SI model 67) by a scrubber of crystals of ferrous sulphate heptahydrate.

As the exposure chamber was in a laboratory, in an air-conditioned building without externally opening windows, we assumed that the temperature and humidity varied little from day to day, and there was negligible particulate pollution. Since the atmospheric levels of SO_2 and O_3 were below 0.1 p.p.m. at the time of exposures, the contamination of the experimental atmosphere was unlikely.

All experiments followed the same protocol. Control pulmonary function was tested at time zero, and subjects then exercised on a bicycle ergometer at a work rate sufficient to double their ventilation for 15-min periods, alternating with 15-min rest periods for 2 h. They were tested again at 30-min intervals from the onset of exposure (0.5, 1.0, 1.5, 2.0 h) with a final test 30 min after the end of exposure.

Forced expiratory flow–volume curves were recorded by a Fleisch No. 3 pneumotachograph connected to a differential pressure transducer (HP model 270) and coupled to a carrier preamplifier (HP model 8805A). The resulting flow signal was displayed against volume on a storage oscilloscope equipped with dual trace amplifiers, volume being obtained by integration of flow signal.

This volume signal was recorded simultaneously on a four-channel FM tape recorder for later tracing of a spirogram. Four flow–volume curves were recorded on each occasion, and the definitive value was taken as the mean of the two largest values of the four. In addition to the dynamic lung function tests, six slow vital capacity curves were measured, three before and three after the exposure.

We have already described the effect of 0.37 p.p.m. of ozone

on human pulmonary function, finding a just significant decrease in maximal mid-expiratory flow rate (MMFR) at the end of 2 h (ref. 3). We now report that SO_2 , when present alone at a concentration of 0.37 p.p.m. had no significant effect on ventilatory function in 2 h. These results are shown in Fig. 1, which also shows the decline in this measurement of ventilatory function when both gases were present together. Clearly, at concentrations of this magnitude, the two gases together have a much greater effect than does either individually.

As in previous tests of human response to air pollutants, there was considerable individual variation. The largest decrease in maximal mid-expiratory flow rate was from an initial value of 7.93 l s^{-1} to 2.78 l s^{-1} at the end of 2 h, and the smallest reduction was from a control value of 8.74 l s^{-1} to 7.98 l s^{-1} after 2 h. For the group as a whole, the change after 30 min of exposure (from a mean of 6.49 with s.d. of ± 1.72 to a mean of 5.60 with s.d. of ± 1.63) was already significant at the 2% level. Other measurements of ventilatory function computed from either expiratory flow–volume curves or spiograms also decreased progressively during the same 2-h experiments.

The forced expiratory volume in 1 s ($\text{FEV}_{1.0}$) decreased to 78% of its initial value at the end of 2 h when both gases were present; the mid-expiratory flow rate, at 50% of vital capacity, decreased to 54% of its initial value in this atmosphere and the peak expiratory flow rate decreased to 79% of its initial value. The vital capacity, measured as a static, non-forced parameter of function, was 92% of its initial value at the end of 2 h exposure for the group as a whole. The decrease in the flow measurements was thus proportionately much greater than the decrease in static volume.

The enhancement of the toxicity of SO_2 and O_3 , present together, has been known for plants for some years⁴. As the simplest approximation, one can imagine the two gases interacting rapidly in the humidified air of the airways and on the large, wet air–tissue interface of the lung, painting the inner surface with sulphuric acid.

Are these experiments reliable indicators of what may be happening outside the laboratory? Lebowitz *et al.*⁵ have demonstrated a decline in ventilatory function in children and adolescents after outdoor exercise on days of relatively high pollution in Tucson, Arizona. Their air pollution data suggest that the interaction of oxidants and sulphur dioxide and sulphate produce the effect, since the levels of nitrogen dioxide and particles were not high enough to cause decline in function.

Are these experiments useful indicators of acceptable and unacceptable environments? At the very least, we can conclude that it is unwise to consider quantitatively acceptable levels for single pollutants when synergism among various pollutants may occur. Nevertheless, in our view, those environments with high enough levels of mixed pollutants to cause discomfort or even adverse changes in pulmonary function on moderate physical activity, should not be acceptable. Furthermore, we can safely assume that the long-term consequences of such repetitive insults may well result in serious lung damage.

M. HAZUCHA*

Department of Physiology, McGill University,
Montreal, Quebec, Canada H3G 1Y6

D. V. BATES

Faculty of Medicine, University of British Columbia,
Vancouver, British Columbia, Canada V6T 1W5

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*To whom reprint requests should be addressed.

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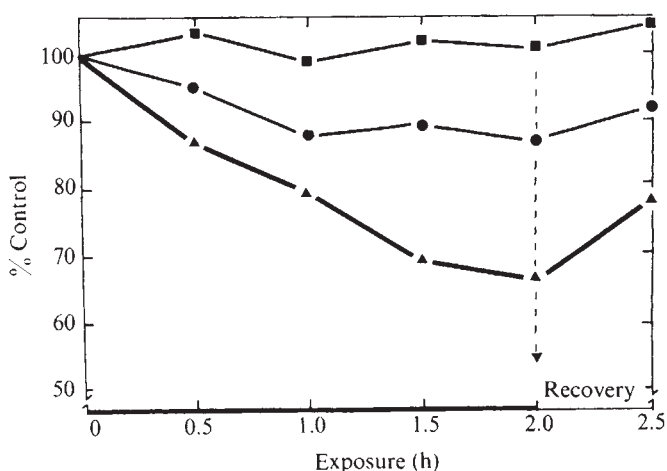
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Fig. 1 Effect of SO_2 and O_3 , separately and together, on maximal mid-expiratory flow rate in intermittently exercising non-smokers. The arrow indicates the beginning of the recovery period. ■, 0.37 p.p.m. SO_2 ($n = 4$); ●, 0.37 p.p.m. O_3 ($n = 8$); ▲, 0.37 p.p.m. $\text{SO}_2 + \text{O}_3$ ($n = 8$).



Immunologically-mediated restraint of latent tumour metastases

DIFFERENT grafted sarcomas, all originally from chemically-induced autochthonous tumours, vary widely in their capacity to form metastases, as determined by the incidence of secondaries which appear in the lung and lymph nodes in the months following surgical removal of the 'primary' transplant from syngeneic rats¹. Tumours which do not form metastases readily in normal animals can be induced to do so by grafting into syngeneic rats depleted of T lymphocytes by procedures which do not compromise the bone marrow¹. We concluded that specific immune processes requiring the participation of T lymphocytes contributed to the control of metastatic spread—possibly by reducing the number of tumour cells released because there is an inverse relationship between the number of macrophages within the tumour and metastasis^{1,2}. In addition, circulating tumour cells may be destroyed immunologically and an immunologically-specific factor that acted against intravenously-injected sarcoma cells was demonstrated in the serum and lymph of tumour-bearing rats^{3,4}.

The experiments which we now report illustrate a further mechanism by which host immunity may control the development of distant metastases. Pure-line Hooded rats received transplants of the syngeneic 3,4-benz-pyrene-induced sarcoma HSBPA into the left leg, and 2 weeks later the tumours and their local draining lymph nodes were removed by amputation of the whole limb as described previously¹. Figure 1 shows that approximately 10% of the animals so treated died of distant metastases 55–100 d after removal of the tumour, the rest remaining tumour-free during the observation period of 18 months (that is, more than half of their normal lifespan). If the rats were exposed to either whole-body X irradiation, or to 7 d continuous draining of the thoracic duct lymph at different times after the removal of the primary transplant (the longest interval so far studied being 1 month), however, there was a highly significant increase in the incidence of distant metastases compared with the control group. In addition, it was noted that individual animals had more tumour lesions in both lymph nodes and lungs than control animals, and that their deaths due

to metastases frequently occurred earlier—especially in groups treated soon after surgery.

Although both whole-body irradiation and severe lymph depletion affect many different aspects of the animal's physiology, their main common feature is that of suppression of lymphocyte-mediated immunity, and it seems likely that this factor is responsible for their similar effects on the growth of metastases. Reconstitution experiments to determine whether the induction of metastases by these procedures can be reversed by giving syngeneic lymphocytes are in progress.

The fact that immunosuppressive treatments, even when given 1 month after surgical removal of a tumour, increase the incidence of metastases, excludes the possibility that in this case we are dealing with an effect on circulating tumour cells. We conclude that even sarcomas which normally are essentially non-metastatic shed cells which lodge and survive in the lung and lymph nodes, where they remain in a viable state without growing into macroscopically detectable lesions. Immunosuppression of the host permits such cells to develop into lethal tumours. This observation does not preclude some form of immunological control before these cells lodge themselves in the lung and lymph nodes, as the incidence of metastases reported here for postsurgically-immunosuppressed animals is not as high as that reported previously for the same tumour when immune suppression was applied during the period of tumour growth¹.

The existence of dormant cells which retain the potential to grow in rats from which the primary transplants have been surgically removed is paradoxical, as these same rats are capable of rejecting inocula of up to 10^7 cells given intramuscularly¹. Indeed, the claim that the tumour is highly immunogenic rests on this finding. We are now attempting to discover if these latent metastases persist as single cells, avascular clusters, or microfoci which are undergoing cyclical growth and regression. One fact seems clear—that at least half of the animals, seemingly cured surgically, carry dormant tumours that manifest only after treatments which are immunosuppressive. These experiments suggest a cautious approach to the use of chemotherapy for dealing with disseminated cancer cells which may be present after all clinically detectable tumour has been removed by surgery or radiotherapy, as such prophylactic systemic chemotherapy is often immunosuppressive. Prophylactic chemo-

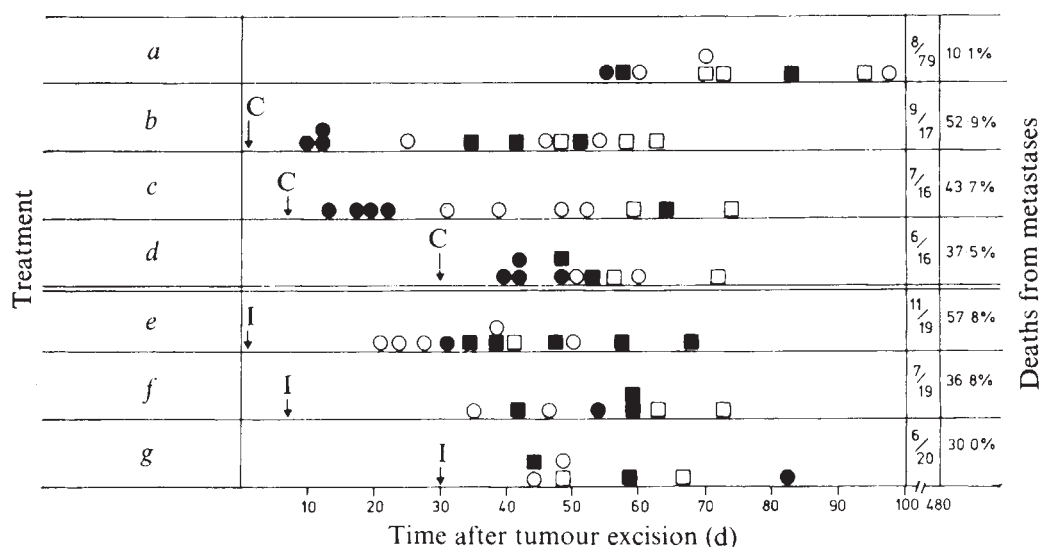


Fig. 1 Effect of postsurgical immunosuppression on incidence of metastases after excision of HSBPA tumour. HSBPA tumours were grown intramuscularly in hind limbs of Hooded rats for 14 d, and removed by amputation of the whole limb. Immunosuppressive procedures were initiated 1, 7 or 30 d after tumour excision. These were: chronic thoracic duct lymph drainage (lymph was drained for 7 d during which time $1-3 \times 10^6$ lymphocytes were removed, and the cannulae were then tied off); and whole-body irradiation (rats were given

one sublethal dose of 500 rad X irradiation from a conventional Marconi machine at 220 kV, 15 mA and 54 cm FSD). a, No treatment (controls; $n = 80$); b-d, thoracic duct lymph drainage ($n = 20$ in each case); b, days 1-7; c, days 7-14; d, days 30-37; e-g, whole-body irradiation: e, day 1; f, day 7; g, day 30. Deaths: ●, with no evidence of metastases; ○, with lymph node metastases; ■, with lung metastases; □, with lymph node and lung metastases. C, cannulation; I, irradiation carried out on days indicated by arrows.

therapy is clearly of value when progressive microlesions are present, which if not eradicated would definitely develop into overt metastases; but it may be harmful when the disseminated tumour cells are dormant.

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SUZANNE A. ECCLES
P. ALEXANDER

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Stimulation of hepatocytes and lymphocytes *in vitro* by liver regeneration

WE have reported the presence of serum factor triggering DNA synthesis in partially hepatectomised rats, using a parabiosis with direct aortic cross circulation¹. The finding was subsequently confirmed with experiments both *in vivo*² and *in vitro*³. We have also reported that partial hepatectomy stimulated function of lymphoid tissues and increased the number of antibody-forming cells in the spleen of rats immunised with sheep red blood cells⁴. It was then not certain how hepatectomy modifies the immune reaction, but now we present evidence that the humoral factor stimulates both hepatocytes and lymphocytes in culture.

Male Lewis rats (80–100 g) were subjected to 70% partial hepatectomy under ether anaesthesia in the morning⁵. Sham operation consisted of an abdominal incision and gentle palpation of the liver. All rats were anaesthetised again with ether 8 h after operation and were exsanguinated by cardiac puncture. Pooled blood was stored overnight at 4 °C, and then the serum was obtained by centrifugation. The serum was kept frozen at –20 °C until used.

Primary liver cell cultures were derived from livers of Lewis rat foetuses at 18–20 d gestation. Single cell suspensions were prepared after collagenase digestion of the tissue⁶. The viability of the cells was 85%, as measured by the Trypan blue exclusion test. The cells were grown in Falcon plastic dishes (35 mm) containing Eagle's MEM which was supplemented with non-essential amino acids, antibiotics, and 10% heat-inactivated foetal calf serum (Grand Island Biological). The initial cell inoculum was 5×10^5 per 2 ml per dish and maintained at 37 °C in a 10% CO₂-air incubator. The medium was changed 24 and 48 h after plating. At 4 d, the old medium was removed from the cultures and the dishes were washed with serum-free medium. Fresh medium (2 ml) containing the serum to be tested at a final concentration of 10%, was added to the culture along with ³H-thymidine (2.5 µCi ml⁻¹). After incubation at 37 °C for 24 h, the medium was removed and the dishes washed twice with Tris buffered saline (pH 7.4). A sample (2 ml) of a 0.05% trypsin solution in Ca²⁺- and Mg-free Tris buffer

were added to the dish and incubated at 37 °C for 30 min. The resulting cell suspension was used to determine the cell number and radioactivity of acid-precipitable material in a Packard scintillation counter. Identical experiments were performed with the foetal kidney cell culture.

To test the effect of partially hepatectomised serum on lymphocyte replication, lymphocyte suspensions were made from the cervical and mesenteric lymph nodes of Lewis rats. The culture medium used was the same for the foetal liver cell culture except that 10% heat inactivated rat serum (pooled from BN strain) was used instead of the foetal calf serum. To show that the 10% foetal calf serum was mitogenic to the rat lymphocyte in our hands, it was substituted for the rat serum which gave a low control value. The initial suspension contained 1×10^6 cells per 2 ml in sterile Falcon plastic culture tubes. The cells were cultured undisturbed for 4 d and supplemented with fresh medium containing the serum in question as mentioned above. At the end of the culture (day 5) smears of cell suspensions were made and stained with Wright–Giemsa. The percentage of mitoses was determined in counts of 1,000 cells. DNA synthesis was also estimated in all experiments by the incorporation of ³H-thymidine.

Results of the ³H-thymidine incorporation into liver cells and lymphocytes are shown in Table 1. A marked increase of DNA synthesis was seen in the liver cells cultured with partially hepatectomised (PH) serum (Table 1, left column). Serum taken from either sham-operated syngeneic (Lewis) or allogeneic (BN) rats was also shown to be mitogenic to Lewis foetal liver cells, compared with the cells cultured without the rat serum. The results strongly favour the hypothesis that partial hepatectomy increased mitogenic serum factor³ but does not decrease inhibitor. No mitogenic effect of the PH serum was observed on the kidney cell culture (data not shown).

Surprisingly the PH serum was also stimulatory to lymphocytes (Table 1, right column). This effect is highly significant compared with either normal rat serum ($P < 0.001$) or sham-operated rat serum ($P < 0.001$). All three sera (NR, PH, and sham) showed significantly high uptake when compared with the culture without the serum. The stimulatory effect of the hepatectomised serum on lymphocyte replication was first seen after the cells were cultured for 8 h or more.

The number of cells in mitosis is shown in Table 2. The serum from partially hepatectomised rats stimulated mitosis of both liver cells and lymphocytes in culture significantly more ($P < 0.001$) than the sera from either normal or sham-operated rats.

Evidence suggesting the presence of regeneration factor in the serum of partially hepatectomised rats was first provided by Bucher *et al.*⁶ in experiments with simple parabiotic rats connected only with skin and muscles. It was firmly confirmed by a cross circulation of aortae of parabiotic rats; a significant increase in DNA synthesis in the normal liver of a parabiotic partner after a 70% hepatectomy of the other^{1,2}. Stimulating effect of the regeneration serum on the growth of fibroblasts⁷, cultured rat hepatocytes⁸ and foetal liver cells⁹ has since been reported.

Table 1 Stimulation of DNA synthesis as measured by incorporation of ³H-thymidine into hepatocytes and lymphocytes *in vitro* in the presence of serum from partially hepatectomised rats

Experiment	Foetal liver cells (c.p.m. per 0.5×10^6 cells per 24 h)				Lymphocytes (c.p.m. per 10^6 cells per 24 h)			
	NR serum	PH serum	Sham serum	No serum	NR serum	PH serum	Sham serum	No serum
1	6,350	13,100	5,190	2,380	2,000	5,130	1,750	1,060
2	9,100	17,500	6,500	3,590	2,150	4,200	2,010	870
3	5,300	11,000	5,900	4,070	1,560	3,950	1,310	690
4	6,100	9,500	4,850	3,410	1,900	4,100	1,550	930
5	5,050	12,600	3,760	1,820	1,610	3,500	1,600	770

Liver cells (left column) were cultured in 35-mm plastic dishes in 2 ml of Eagle's medium supplemented with 10% heat-inactivated foetal calf serum. On day 4, the old medium was pipetted and the cultures incubated for 24 h with 2 ml of medium containing either 10% normal rat (NR), partially hepatectomised (PH), sham-operated rat sera or no serum along with ³H-thymidine (2.5 µCi ml⁻¹). Lymph node lymphocytes (right column) were cultured in 5-ml plastic tubes in 2 ml of the medium with 10% heat-inactivated pooled homologous serum. Addition of the serum to be tested and assay for DNA synthesis were the same. Each experiment was done on 3 rats. Average of 3 dishes is shown for each experiment.

Table 2 The mitotic rate of hepatocytes and lymphocytes cultured with partially hepatectomised rat serum for 24 h.

Serum source	Mitosis (% mean $\pm$ s.d.)	
	Foetal liver cells	Lymphocytes
Normal rats	4.83 $\pm$ 2.01	0.28 $\pm$ 0.16
Partially hepatectomised rats	9.05 $\pm$ 2.64	0.87 $\pm$ 0.20
Sham-operated rats	4.29 $\pm$ 1.87	0.31 $\pm$ 0.15
None	3.56 $\pm$ 1.08	0.12 $\pm$ 0.08

Culture conditions for both liver cells and lymphocytes are the same as shown in Table 1. At the end of the culture (on the day 5) smears of the cell suspensions were made and stained with Wright-Giemsa. The percentage of the mitosis was determined per 1,000 cells. The data represent means with standard deviations from total 15 cultures for each serum tested.

Partial hepatectomy leads to increased DNA synthesis not only in the regenerating liver cells but also in lymphoid tissues as measured by ^3H -thymidine uptake⁹. We have shown that partial hepatectomy affects the recruitment of thymus cells on the one hand, but on the other, it increases the number of antibody-forming cells by shortening their doubling time^{4,10}. It was then suspected that the regeneration serum might be mitogenic to both the hepatocyte and lymphocyte. For a technical reason in the present experiment, both types of cells, foetal liver cells and adult lymph node cells were treated slightly differently in that the former were cultured after collagenase digestion of the tissue and in a 10% foetal calf serum medium, whereas the latter were cultured in a 10% rat serum medium without the enzymatic digestion. While it might be argued that the enzyme and different serum sources might have triggered the membrane receptors of both cell types differently, these culture conditions would not be a decisive factor, since only the PH serum could stimulate the DNA synthesis of both cells when added at the end of the fourth day and cultured more than 8 h.

The breakdown of the peripheral lymphocytes took place 7-8 h after partial hepatectomy and the peak of RNA synthesis in the liver appeared at the same time. It is therefore possible that the breakdown product of the lymphocyte liberates other active agents independent of hepatotrophic factor, which stimulate increased precursor cell proliferation of the lymphocyte. Although the fact that the PH serum did not stimulate the cultured kidney cells suggests a hepatolymphatic inter-relationship, it remains to be seen whether the serum factor(s) appearing after partial hepatectomy is multipotential, one factor triggering the hepatocyte replication and the other stimulating the lymphocyte, or unipotential, a factor regulating both cell types.

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A. SAKAI
S. L. KOUNTZ

Department of Surgery,
Downstate Medical Center,
State University of New York,
Brooklyn, New York 11203

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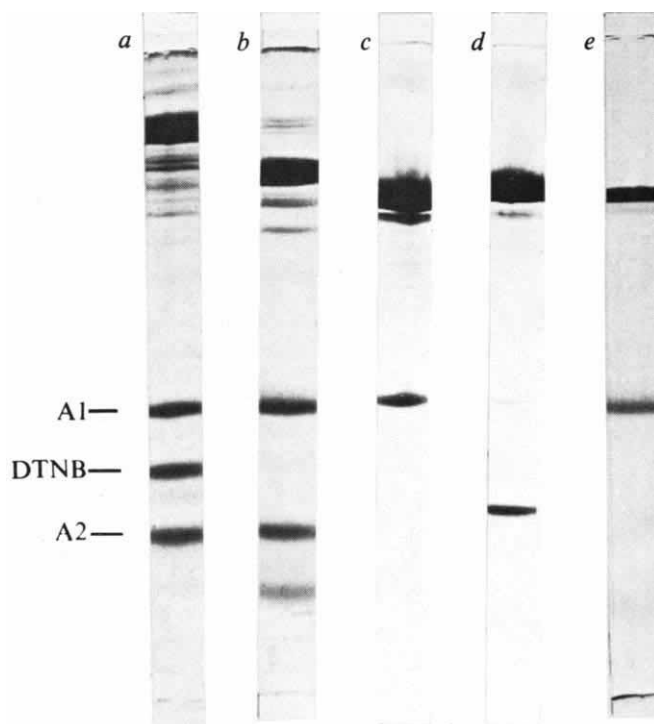
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Separation of subfragment-1 isoenzymes from rabbit skeletal muscle myosin

RABBIT skeletal muscle myosin is composed of two heavy chains and four light chains¹. Two classes of light chains can be distinguished both chemically and functionally. Within the class of essential light chains (those which cannot be removed without loss of ATPase activity), two chemically related but phenotypically distinct proteins have been identified², both of which are present in single fibres of fast-twitch muscles³. The existence of both phenotypes within a single psoas muscle cell indicates that either there is a single population of myosin molecules with a different alkali light chain on each subfragment-1 head, or there are at least two populations of myosin present. Densitometric and radiochemical methods have shown that there is an unequal distribution of these two light chains; this supports the hypothesis that myosin isoenzymes occur in histochemically homogeneous muscles³⁻⁵ which cannot be attributed to the presence of contaminating slow-twitch fibres. The presence of different heavy chains also is indicated by the observation of amino acid substitutions in certain peptide sequences^{6,7}. Thus fast-twitch muscles contain myosin showing heterogeneity of both light and heavy chains, suggesting isoenzyme populations, but so far these have not been separated, nor have the proteolytic subfragments derived from them.

The smallest active fragment obtainable from myosin is the globular head called subfragment-1 (S-1), which is usually produced by brief papain digestion⁸. This subfragment has been used extensively for kinetic analysis of the ATPase activity⁹. Unfortunately it is even more heterogeneous than its parent myosin. Partial loss of the non-essential DTNB light chain is commonly observed on exposure of myosin to papain⁸, and the

Fig. 1 Polyacrylamide gel electrophoresis on 10% gels run in 0.1 M Tris-bicene and 0.1% SDS and stained as described previously³. *a*, Chymotryptic HMM; *b*, chymotryptic S-1; *c*, fraction S-1(A1) pooled from the DEAE-cellulose column; *d*, fraction S-1(A2) from the column; *e*, S-1(A1) from a single peak tube showing one heavy chain and the alkali 1 light chain.



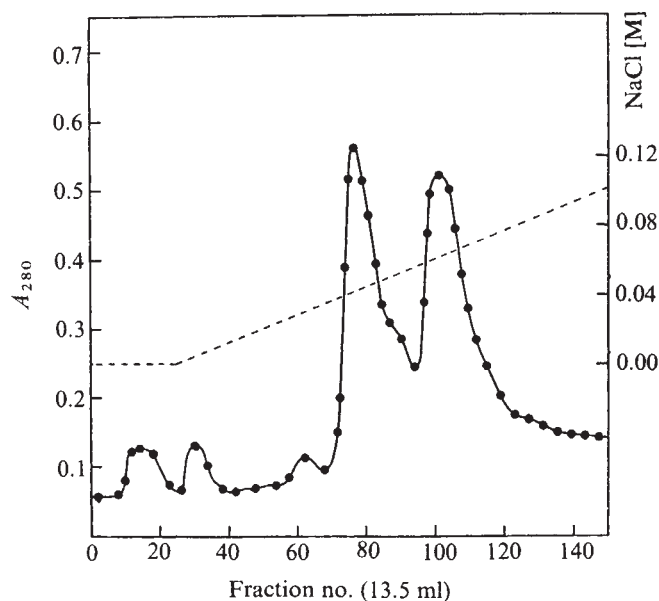


Fig. 2 Chromatography on DEAE-cellulose (Whatman DE-52) was carried out at 4°C using a 48×2.5-cm column in 50 mM imidazole-HCl buffer at pH 7.0. This S-1 was eluted with a linear gradient to 0.12 M NaCl and the absorbance was measured at 280 nm.

heavy chains show the presence of nicks when subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS)^{8,10}.

To characterise the different isoenzymes of myosin it is essential to separate these S-1 fragments, and further to obtain species which do not contain adventitious proteolytic cleavage of the heavy chains. This has not proved possible using papain, but following some observations of Yagi and Otani¹¹, we have developed methods to produce selective cleavage of the myosin head, and have also been able to eliminate the DTNB light chains from the resultant S-1. Two populations of S-1 molecules are obtained; one contains the alkali 1 light chain [S-1(A1)] and the other, alkali 2 [S-1(A2)], and both give single heavy chain bands on polyacrylamide gels. The two populations have been separated by chromatography, modifying the procedure described previously⁸.

Rabbit myosin was prepared as described⁸, and digestion for 10 min at 25°C in 0.6 M NaCl, 10 mM sodium phosphate, pH 7.0, at 10–20 mg ml⁻¹ using α -chymotrypsin at 0.05 mg ml⁻¹ produced heavy meromyosin (HMM), which was isolated in the supernatant after dialysis to an ionic strength less than 0.05. (Digestion was terminated by addition of 100 mM phenyl methane sulphonyl fluoride in ethyl alcohol to a concentration of 10⁻⁴ M.) Gel electrophoresis of this HMM is shown in Fig. 1; it contained a single heavy chain and three light chain bands. If the digestion was carried out on myosin filaments produced by dialysis to 0.12 M NaCl, 20 mM sodium phosphate, pH 7.0, and containing 1 mM EDTA, HMM was no longer produced; instead the product was S-1, which on gels showed a single heavy chain band and only two light chains (Fig. 1). Thus under these conditions the susceptibility of myosin to chymotrypsin is altered, and the S-1 obtained is both more homogeneous and less degraded than that produced by papain digestion.

With the DTNB light chain absent, we have been able to fractionate the two components in this S-1 preparation (Fig. 2). Gel electrophoresis of the products is shown in Fig. 1. Although the second fraction frequently contains some contaminating S-1(A1), the first fraction is remarkably clean, yielding a single heavy chain and one light chain which comigrates with alkali 1 light chain marker. The molecular weights obtained by low speed sedimentation equilibrium for these two components were in the range 116,000–126,000, similar to values obtained for papain S-1 (ref. 8).

ATPase activity was measured to investigate kinetic differences between the two S-1 fractions. The calcium ATPase, measured with a pH-stat, in 30 mM KCl, 10 mM CaCl₂ and 1 mM ATP at pH 8.0, showed no significant differences. For example, in one preparation the turnover number of S-1(A1) was 8.1 s⁻¹ and that of S-1(A2), 8.2 s⁻¹ (both based on a molecular weight of 115,000). In an earlier preparation which was not fractionated, the activity of 7.7 s⁻¹ was identical to that obtained for a parallel preparation of papain S-1.

The magnesium-dependent ATPase activity, measured by a linked assay system¹², again showed no significant differences between these fractions. The steady state rate was always in the range 0.04–0.05 s⁻¹, similar to published values for papain S-1 (ref. 12). When ATPase measurements were made in the presence of actin, striking differences were observed. In three different preparations we found that the V_{max} for S-1(A1) was about half that obtained for S-1(A2). One such experiment is shown in Fig. 3. The regression line extrapolates to give V_{max} = 28 s⁻¹ for S-1(A1), and 54 s⁻¹ for S-1(A2), while K_{app} , determined from the intercept on the abscissa is 10 μ M actin for S-1(A1) and 50 μ M actin for S-1(A2). By comparison, the V_{max} obtained for papain S-1 under these conditions was 40 s⁻¹.

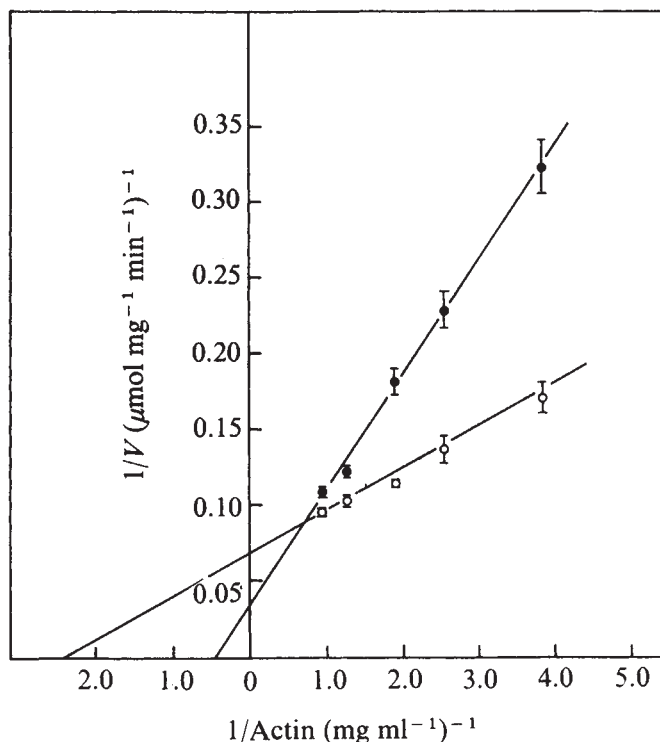


Fig. 3 Lineweaver-Burk plot of actin-activated ATPase activity for S-1(A1) (○) and S-1(A2) (●). The assay mixture contained 6 mM KCl, 1.5 mM MgCl₂ and 1.0 mM ATP, and activities were determined in a pH-stat at pH 8.0, 25°C. The error bars show standard deviations, with at least five determinations at each actin concentration.

We show here that isoenzymes of S-1 obtained by chymotryptic digestion of filamentous myosin, can be separated to give homogeneous S-1 fractions. Steady state ATPase activity measurements showed no significant differences in the absence of actin, while in its presence there was a twofold difference in the V_{max} obtained from Lineweaver-Burk plots. This kinetic variation may reflect a true distinction in the mode of interaction of these different myosin heads with actin, which might be dependent on the presence of different alkali light chains, but it could have arisen as a result of the proteolysis procedures or during purification. Experiments are in progress to explore this. Nevertheless, the results suggest that the structural heterogeneity determines kinetic differences between these S-1 species. It is now important to establish whether the heavy meromyosins

produced by chymotryptic cleavage can be separated, since this would provide unequivocal evidence for the existence of myosin isoenzymes.

The importance of these studies is that using chymotrypsin, homogeneous S-1 preparations can be obtained for the first time. Furthermore, since the specificity of this enzyme is for aromatic residues, which are frequently buried and inaccessible to solvent, this enzyme provides a particularly sensitive probe for the susceptible regions of the myosin molecule: digestion can be modified not only by the aggregation state of myosin but also by its ionic environment, since in the presence of divalent cations (magnesium or calcium), HMM is produced even at low ionic strength (our unpublished observations).

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A. G. WEEDS
R. S. TAYLOR

MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge CB2 2QH, UK

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Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibres

THE occurrence of pleural and peritoneal mesotheliomata is linked with the inhalation of asbestos fibres^{1,2}. Experimentally, mesotheliomata have been induced by the intrapleural inoculation of asbestos fibres into rats³. These findings suggest that

the incorporation of the fibres disturbs processes at the cellular level. The possibility that they induce chromosome abnormalities was indicated by V. Timbrell (personal communication) and we have investigated it further using cultured Chinese hamster cells.

The cells were derived by cloning from a parental line CHO-K1 (American Type Culture Collection), a line which has previously been used in evaluating induced chromosome changes⁴. They were seeded at equal densities in 10 culture vessels containing Iwakata and Grace's modification of McCoy's 5a culture medium⁵ (foetal bovine serum level 10%). Pairs of flasks, with the exception of two unexposed control cultures, contained one of the following dusts at a concentration of 0.01 mg ml⁻¹, SFA chrysotile asbestos; UICC crocidolite asbestos; glassfibre and glass powder. These dusts have been described elsewhere⁶. The flasks were agitated to mix the cells and dusts thoroughly and after 48 h the cells from one member of each pair were collected, metaphase spreads being obtained by the standard procedure. Measurements of plating efficiency and viability (by the Trypan blue method) were made for each culture at the time of collection. Five days after seeding, the remaining cultures were processed in the same way. The slides were stained by the trypsin/Leishman banding method of Seabright⁶ and 100 suitable metaphases from each culture were studied.

The experiment was then repeated with the notable difference that the cells were treated after culture by a method of *in situ* fixation (to be published elsewhere) which avoided both mechanical handling (that is, centrifugation) and the use of proteolytic enzymes for cell detachment.

The results are summarised in Table 1 and show that karyotypic alterations were largely restricted to the cells exposed to asbestos. Prolonging the exposure period from 48 h to 5 d seems to have had little effect on the incidence of abnormalities, with the possible exception of the 5-d exposed SFA chrysotile group in which there was an increased trend toward cells with fragments. In this context, it is noteworthy that additional experiments in which asbestos fibres were preincubated in culture medium for a short period before coming into contact with cells revealed a reduced incidence of karyotypic abnormality, particularly in the case of the group exposed to UICC crocidolite.

This could indicate that components of the culture medium

Table 1 Effects of different treatments on chromosomes

	48 h exposure				
	SFA chrysotile asbestos	UICC crocidolite asbestos	Glassfibre	Glass powder	Unexposed controls
Polyploids	30 (33)	21 (24)	4 (3)	3 (5)	4 (4)
Cells with fragments	14 (13)	11 (15)	0 (0)	0 (0)	0 (0)
Cells with other chromosomal changes	28 (21)	32 (20)	0 (1)	0 (0)	0 (0)
% Abnormal cells	65 (56)	59 (55)	4 (4)	3 (5)	4 (4)
(100 cells scored from each culture)					
	5 d exposure				
	SFA chrysotile asbestos	UICC crocidolite asbestos	Glassfibre	Glass powder	Unexposed controls
Polyploids	21 (25)	25 (30)	7 (4)	5 (4)	2 (5)
Cells with fragments	34 (31)	14 (10)	0 (0)	0 (0)	0 (0)
Cells with other chromosomal changes	25 (19)	23 (30)	1 (0)	0 (0)	0 (1)
% Abnormal cells	79 (73)	53 (61)	8 (4)	5 (4)	2 (6)
(100 cells scored from each culture)					

The numbers in parentheses refer to results obtained by *in situ* fixation. The categories scored were not mutually exclusive. All the populations showed plating efficiencies > 89% and viabilities > 85% at the times of collection.

(for example, serum protein) interact with asbestos to produce alterations in the effective dose and exposure period within the experiments, such that after a fixed short period fibres were rendered ineffectual with regard to the production of genetic damage. This type of consideration may help to explain the similarity between the 48-h and 5-d exposure data.

The polyploid cells identified were largely cells with twice the modal number of chromosomes in the 48-h exposed groups and more than twice the modal number in the cultures receiving 5-d exposures. The cells classified with fragments (Table 1 and Fig. 2) included many changes at both the chromatid and chromosomal levels; chromatid breaks and paired isodiametric fragments (double minutes) as shown in Fig. 2b. In addition, chromosome type breaks and rearrangements were identified in some cells from the asbestos-treated cultures (Table 1 and Fig. 1), but not from the glass-treated or unexposed controls. The possibility that these may represent the selection of pre-existing clones is being examined in further experiments although this is not supported by ancillary evidence.

The inclusion of fibreglass and glass powder in the experiment as presumptively inert control dusts was prompted by the finding that, in contrast to asbestos fibres, neither gives rise to mesotheliomata when inoculated intrapleurally into rats³. It is noteworthy that polyploids were for the most part the only exceptional cells identified in the cultures exposed to these two dusts and that their incidence was no greater than in the unexposed controls. In the absence of other changes in the glass-exposed cultures it seems unlikely that the chromosomal abnormalities seen in the cells exposed to asbestos arose as a result of the mechanical handling (centrifugation) that followed cell culture. This is further confirmed by the results obtained

Fig. 1 *a*, Karyotype of unexposed cell. *b*, karyotype of cell exposed for 5 d to UICC crocidolite asbestos. Arrows indicate chromosomes that have undergone pericentric inversion. Chromosome 16 has broken in the region of its centromere and marker chromosome 20 is absent.

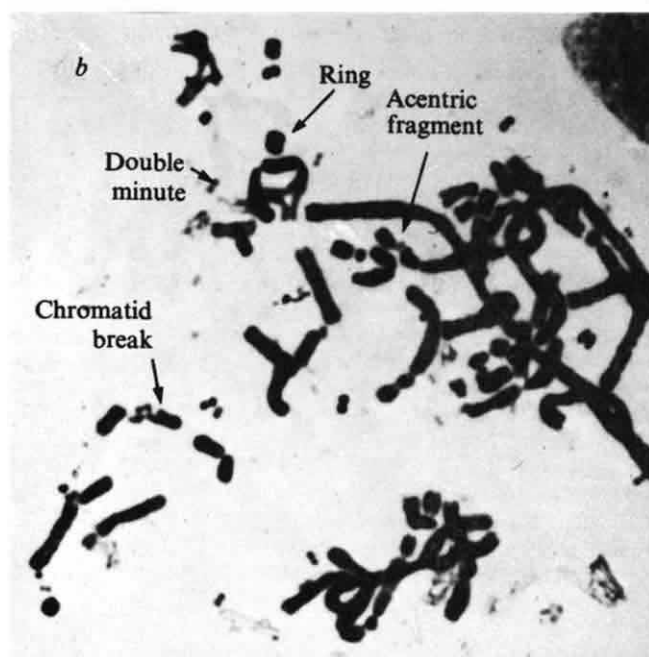
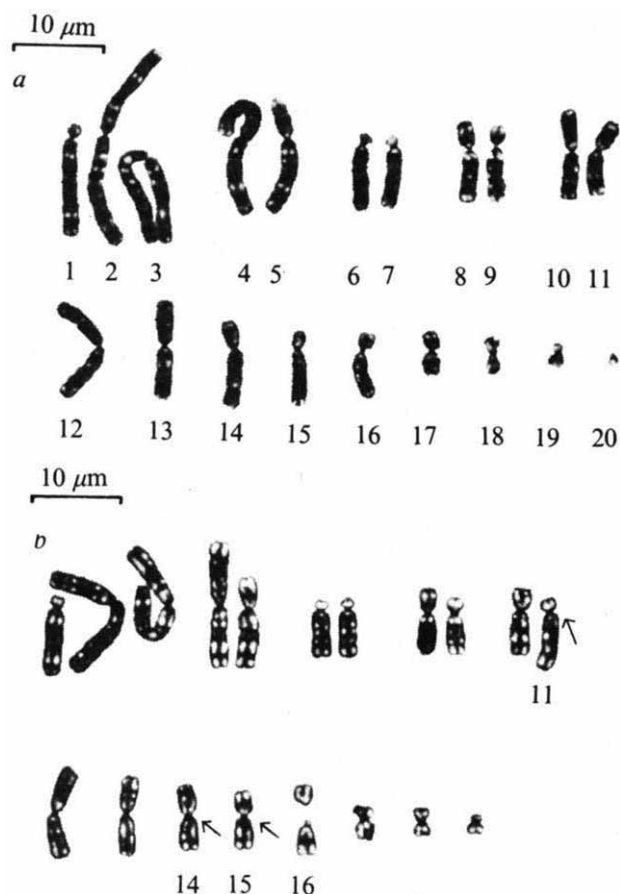


Fig. 2 *a*, Cell derived from a culture that had been exposed to UICC crocidolite asbestos for 5 d, showing multiple fragmentation in a situation of high ploidy. A fibre is seen at the centre of the field. *b*, Chromatid breaks and multiple aberrations in a cell derived from a culture exposed to UICC crocidolite asbestos for 5 d.

using an *in situ* fixation method (Table 1) which correlate closely with those obtained using standard procedures, although the method avoids both centrifugation and the use of proteolytic enzymes for cell detachment.

We conclude that multiple abnormalities at both the chromatid and chromosomal levels were produced in Chinese hamster cells as a result of the inclusion of asbestos fibre in their culture regime. Furthermore, these results, which were specifically produced by asbestos rather than the presumptively inert glass dusts, seemed to be largely unaffected by prolonging the exposure period. This preliminary detection of a mammalian cell response to asbestos *in vitro* forms the first stage of the development of an *in vitro* screening test for asbestos and other carcinogenic materials. In this it is envisaged that primary cells

will be cloned for specific chromosomal abnormalities and then introduced into laboratory animals to ascertain whether or not they induce tumours *in vivo*.

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ANDREW SINCOCK

MRC Pneumoconiosis Unit,
Llandough Hospital,
Penarth, Glamorgan CF6 1XW, UK

MARINA SEABRIGHT

Wessex Regional Cytogenetics Unit,
Salisbury General Hospital,
Salisbury, Wiltshire, UK

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Cyclic AMP-mediated transformation of rat cells transformed by temperature-sensitive mouse sarcoma virus

SEVERAL lines of evidence suggest that adenosine 3',5'-cyclic monophosphate (cyclic AMP) is involved in the regulation of growth and other properties of cultured cells (for review, see ref. 1). Specifically, various reports have shown a correlation between the abnormal growth and morphological properties of virus-transformed cells and low levels of cyclic AMP^{2,3}. Treatment of these transformed cells with cyclic AMP, cyclic AMP analogues, or agents that raise cyclic AMP levels restores growth and morphological properties characteristic of normal cells⁴⁻⁷. The use of cells transformed by temperature-sensitive (ts) mutants of Rous sarcoma virus (RSV) or mouse sarcoma virus (MSV)^{8,9} as well as ts host cell mutants^{10,11} strengthened the evidence relating viral transformation and cyclic AMP levels. Other evidence obtained in similar virally transformed cell systems, however, has failed to confirm the inverse relationship between cyclic AMP levels and cell growth rate and transformation by RNA and DNA tumour viruses¹²⁻¹⁵. We now report that the exogenous addition of cyclic AMP mediates the morphological transformation of rat cells transformed by a ts mutant of MSV at the non-permissive temperature. Correspondingly, expression of the transformed phenotype by cells grown at the permissive temperature is associated with increased levels of endogenous cyclic AMP.

The cell lines used have been described before^{16,17} and included: (1) normal rat kidney (NRK) cells, (2) NRK cells transformed and productively infected with the Moloney sarcoma-leukaemia virus (NRK(MSV-MLV)), and (3) NRK cells transformed by a cold-sensitive mutant of MSV (NRK(MSV-1b)). The NRK(MSV-1b) cells express the transformed phenotype at the permissive temperature (39 °C) but appear phenotypically normal at the non-permissive temperature (33 °C). Cell lines other than NRK(MSV-1b) were routinely grown at 36 °C.

Preliminary experiments to investigate the effect of cyclic AMP on the morphological phenotype of uninfected and MSV-transformed NRK cells, demonstrated that 0.4 mM 8-bromo-cyclic AMP (8-Br-cyclic AMP) or 0.4 mM N⁶,O^{2'}-dibutyryl cyclic AMP (db-cyclic AMP) in combination with 1.0 mM theophylline were most effective in inducing the morphological changes presented in Fig. 1. The morphology of NRK(MSV-1b) grown at the non-permissive temperature (Fig. 1a) was changed perceptibly after exposure to 8-Br-cyclic AMP or db-cyclic AMP (Fig. 1b). The effect was to change the morphological phenotype of NRK(MSV-1b) cells

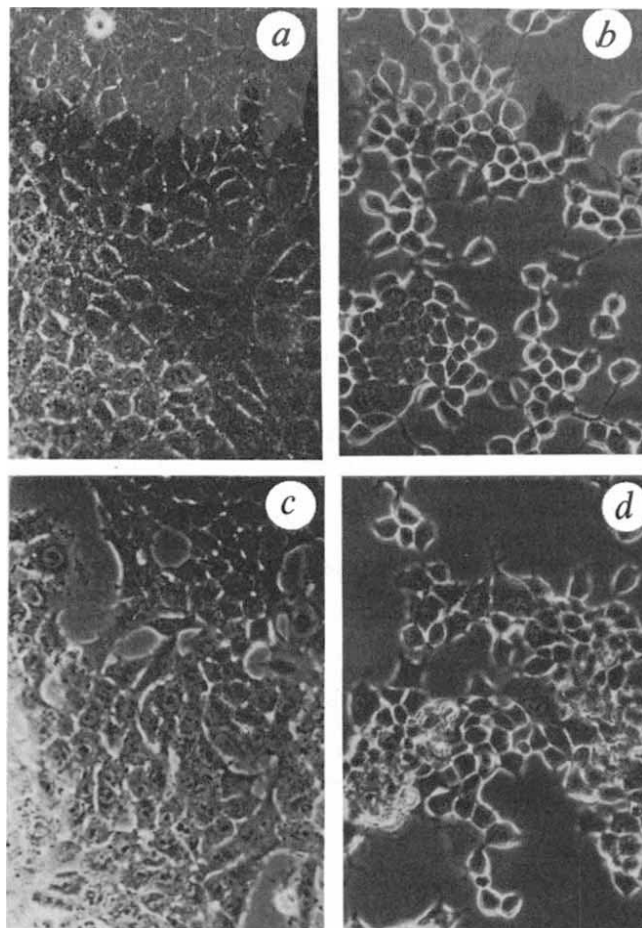


Fig. 1 Effect of cyclic AMP on the morphology of NRK(MSV—1b) cells. NRK(MSV—1b) cells were seeded at 1×10^6 per 60-mm Petri dish and incubated at either 33 or 39 °C for 3–4 d in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum (v/v). They were then exposed to 0.4 mM db-cyclic AMP plus 1.0 mM theophylline or 0.4 mM 8-Br-cyclic AMP without replacing the growth medium. Twenty-four hours later photomicrographs were made with a phase-contrast microscope and were magnified $\times 190$. Solutions of cyclic AMP, cyclic AMP analogues, other cyclic nucleotides, adenosine compounds and theophylline in growth medium were prepared and Millipore-filtered (0.45 μ m) immediately before use. *a*, NRK(MSV—1b) cells grown at the non-permissive temperature, 33 °C, and mock-treated with growth medium; *b*, NRK(MSV—1b) cells grown at 33 °C and exposed to 0.4 mM db-cyclic AMP plus 1.0 mM theophylline for 24 h; *c*, cells treated as in *b*, reversed at 24 h by replacing the medium with growth medium, and photographed 2 h later; *d*, NRK(MSV—1b) cells grown at the permissive temperature, 39 °C, and either mock-treated with growth medium, or exposed to 0.4 mM db-cyclic AMP plus 1.0 mM theophylline for 24 h. Identical results were obtained using 8-Br-cyclic AMP in place of db-cyclic AMP and theophylline.

which at the non-permissive temperature, 33 °C, appear flattened and poorly refractile to a highly refractile, rounded, circular morphology, characteristic of NRK(MSV-1b) cells grown at the temperature (39 °C) permissive for expression of transformation (Fig. 1d). The morphological change was obvious within 10 min after exposure to 8-Br-cyclic AMP and achieved maximum expression by 1 h. Using db-cyclic AMP plus theophylline, morphological changes were obvious within 3 h and achieved maximum expression by 24 h. In both cases, exposure of NRK(MSV-1b) cells at 33 °C to the cyclic AMP analogues resulted in morphological alterations of 100% of the cells, and the cells remained morphologically altered as long as additive was present (up to 72 h). Removal of the cyclic AMP analogues, however, resulted in a rapid reversal (within 1 h) from the transformed to the normal phenotype (Fig. 1c). Furthermore, the morphological changes induced

by cyclic AMP were apparently independent of the growth state of the NRK(MSV-1b) cells, since identical morphological responses were observed in sparse and confluent cultures. The addition of cyclic AMP analogues to NRK(MSV-1b) cells grown at the permissive temperature (39 °C) did not alter the existing transformed phenotype characterised by rounded cells that grew in clumps or clusters. The exogenous addition of cyclic AMP analogues to uninfected NRK cells and transformed NRK(MSV-MLV) cells provoked no morphological changes in these cells whether cultured at 33, 36 or 39 °C, indicating that the cyclic AMP-mediated morphological changes were specific for NRK cells transformed by the cold-sensitive MSV mutant.

The specificity of cyclic AMP or analogues of cyclic AMP in inducing morphological transformation of NRK(MSV-1b) cells grown at the non-permissive temperature was investigated. The induced morphological changes were specific for cyclic AMP and cyclic AMP analogues and potentiated by theophylline. Cyclic GMP alone had no effect on the morphology of NRK(MSV-1b) cells and was not antagonistic to the effects of the cyclic AMP analogues. Molecules related to cyclic AMP, such as 5'-adenosine monophosphate (5'-AMP), 2',3'-AMP, adenosine, and adenine had no effect on change of cell shape. Similarly, other cyclic nucleotides such as cytidine 3',5'-cyclic monophosphate (cyclic AMP), thymidine 3',5'-cyclic monophosphate (cyclic TMP), uridine 3',5'-cyclic monophosphate (cyclic UMP), as well as theophylline and butyrate had no effect on the morphological phenotype of the cells.

One prediction of the cyclic AMP-mediated morphological transformation event would propose that intracellular cyclic AMP levels should be lower in the phenotypically normal cold-sensitive transformants grown at the non-permissive temperature, whereas higher levels of cyclic AMP might be expected in the same cells grown at the permissive temperature where the transformed phenotype is expressed. To test this prediction, intracellular cyclic AMP content was measured by a standard radioimmunoassay (Collaborative Research) as previously described¹⁸. NRK(MSV-1b) cells grown under permissive conditions contained levels of cyclic AMP approximately three times higher than the same cells grown under non-permissive conditions (Table 1). Levels of cyclic AMP remained 50–80% higher in cells grown at the permissive relative to the non-permissive temperature during the course of the experiment. Reciprocal temperature shifts of NRK(MSV-1b) cells grown at 39 °C or 33 °C resulted in marked changes in cyclic AMP levels (Table 1). Cyclic AMP levels increased approximately threefold within 5 h after a shift from non-permissive to permissive temperature and reached a level 24 h later similar to that of cells grown at 39 °C. In shift-down experiments, cyclic AMP levels fell more slowly with time, resulting in a 50% decrease within 24 h after shifting from the permissive to non-permissive temperature. The slower drop in cyclic AMP levels contingent with shifting from 39 to 33 °C may reflect the time involved in turnover of the residual cyclic AMP pool. Alternatively, the rate at which cyclic AMP levels decrease or increase after temperature shift may be controlled by temperature-dependent adenylate cyclase activity.

During these experiments, uninfected NRK and transformed NRK(MSV-MLV) cell lines were included at random in the experimental design and their cyclic AMP levels are presented in Table 1. No significant difference in cyclic AMP levels was found for NRK and NRK(MSV-MLV) cell lines under the conditions described.

Our observations of higher levels of cyclic AMP in NRK(MSV-1b) cells grown at the temperature permissive for expression of the transformed phenotype relative to the same cells grown under conditions non-permissive for maintenance of transformation parallels the morphological changes that occur after addition of cyclic AMP or cyclic AMP analogues to these cells. Recently, we have examined fucosylglycolipid metabolism as a biochemical marker of transformation²⁰.

Table 1 Cyclic AMP levels in NRK cells transformed by temperature-sensitive MSV

Expt	Time	pmol cyclicAMP per mg protein			
		NRK NRK (MSV-MLV)			
		NRK(MSV-1b)			
		33 °C	33→39 °C	39 °C	39→33 °C
1	0 h	28*	28	90	90
	5 h	41	111	63	80
	24 h	39	69	69	59
2	5 d			61	51

In experiment 1 NRK(MSV-1b) cells were seeded at 1.5×10^5 cells per Petri dish and incubated at 33 or 39 °C. Forty-eight hours later, half of the dishes at 39 °C were shifted to 33 °C, and vice versa. At the time of temperature shift (0 time), 5 h and 24 h later cyclic AMP content was determined. Medium was aspirated from the cultures, the cells were washed once with cold Gibco Solution A, and the cells were frozen by placing the Petri dishes on dry ice. The cultures were then overlaid with 1.0 ml of cold 6% trichloroacetic acid, and the cells were scraped off the dishes with a rubber policeman. The suspension was centrifuged at 10,000g for 15 min at 4 °C. The precipitate was assayed for protein by the Lowry method¹⁹, and the supernatant was assayed for cyclic AMP. The supernatant was extracted three times with 5 ml of water-saturated petroleum ether. The aqueous phase was heated to 70–80 °C for 2 min and then frozen at –70 °C. In some cases the aqueous phase after extraction was lyophilised and the residue was dissolved in 0.25 M phosphate buffer, pH 7.0. Cyclic AMP was assayed using a standard radioimmunoassay (Collaborative Research) as previously described¹⁸. Recovery of cyclic AMP during extraction was above 95%. In experiment 2 NRK and NRK(MSV-MLV) cells were seeded at 1.5×10^5 cells per Petri dish, incubated at 36 °C, and assayed for intracellular cyclic AMP 5 d later, as described above. The values represent the mean of three independent experiments in which duplicate dishes were assayed in triplicate for cyclic AMP. *Values represent the mean of duplicate dishes. Cyclic AMP determinations were done in triplicate for each sample.

Concomitant with the morphological changes, fucosylglycolipid metabolism was altered markedly after addition of cyclic AMP to the cold-sensitive transformants grown at 33 °C (unpublished results of K.D.S., and S. M. Steiner). It thus seems that this system provides an important exception to mechanisms currently postulated for the role of cyclic AMP in the expression of the transformed state. A solution to the difference between the cyclic AMP-mediated morphological transformation of NRK(MSV-1b) cells and what is usually observed in comparison of cyclic AMP levels and effects in transformed and non-transformed cells awaits the elucidation of the nature of the cold-sensitive transforming function present in cells transformed by the MSV mutant. Perhaps more importantly, the rapid acquisition of the transformed phenotype after exposure to cyclic AMP provides a useful model for studying the control of viral gene expression and should aid in the identification and characterisation of viral gene products responsible for malignant transformation.

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KENNETH D. SOMERS
MARTIN RACHMELER
MARY CHRISTENSEN

Department of Microbiology and Cell Biology,
Eastern Virginia Medical School,
Norfolk, Virginia 23507, and
Department of Microbiology,
Northwestern University Medical School,
Chicago, Illinois 60611

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Genetic heterogeneity in GM1-gangliosidosis

GM1-GANGLIOSIDOSIS is an inherited lysosomal storage disease which is due to a deficiency of the acid hydrolase GM1- β -galactosidase¹. During the past few years several clinical variants have been described²⁻⁶ that differ in time of onset of symptoms, involvement of visceral organs or skeletal tissue and in the degree of neuronal and mental deterioration. Some of these variants have been related to different properties of the deficient β -galactosidases^{5,7} but the significance of the experimental data⁸ has been questioned. Several investigators have speculated on the genetic background of the different variants^{2,8,9} but no experimental evidence has been provided to support the hypotheses.

Using somatic cell hybridisation techniques¹⁰⁻¹² we have investigated whether different gene mutations are involved in clinical variants of GM1-gangliosidosis. Fibroblasts from a patient with the infantile type 1 and the juvenile type 2 (refs 1-3), and from the type 3 patient without mental retardation described by Pinsky *et al.*⁵ were fused with cells from an adult (type 4) patient reported by our group⁶. Two days after hybridisation the occurrence of genetic complementation was tested by β -galactosidase assays with both synthetic and natural substrates. These experiments showed that the gene mutation in types 1 and 2 is different from that in types 3 and 4. Enzyme assays performed in single heterokaryons containing the genomes of type 1 and type 4 cells showed that genetic complementation results in complete restoration of normal enzyme activity.

Normal skin fibroblasts and cells from patients with the different types of GM1-gangliosidosis were grown in Ham F10 medium supplemented with 10% foetal calf serum and antibiotics. In homogenates of fibroblasts from patients with the type 1 or type 2 variant, β -galactosidase activity was less than 1% of control values when measured with the 4-methylumbelliferyl (MU) substrate. Homogenates of fibroblasts from patients with type 3 or type 4 GM1-gangliosidosis had a residual activity of 15-20%. In type 4 cells a 5% and 8% residual activity with the natural substrates GM1-ganglioside and asialofetuin was found by Dr J. S. O'Brien (Department of Neurosciences, University of California, La Jolla).

Cells from the adult type 4 patient were hybridised with those from the other three variants and each cell strain was also fused with itself (parental fusion) as a control. The results of β -galactosidase assays with synthetic substrate are shown in Fig. 1. Levels of activity in unfused cells were similar to those in the parental fusions, which indicated that somatic cell hybridisation did not affect enzyme activity. The β -galactosidase activities in fusions of type 1 $\times$ type 2 and of type 3 $\times$ type 4 cells remain unchanged relative to the average level expected in a mixture of equal numbers of parental cells. Fusion of type 1 $\times$ type 4 cells, however, resulted in a six- to sevenfold increase in enzyme activity. Also there was a markedly increased enzyme activity in fusions of type 4 $\times$ type 2 cells.

Similar results were obtained when the β -galactosidase activity was measured with the natural substrate GM1-

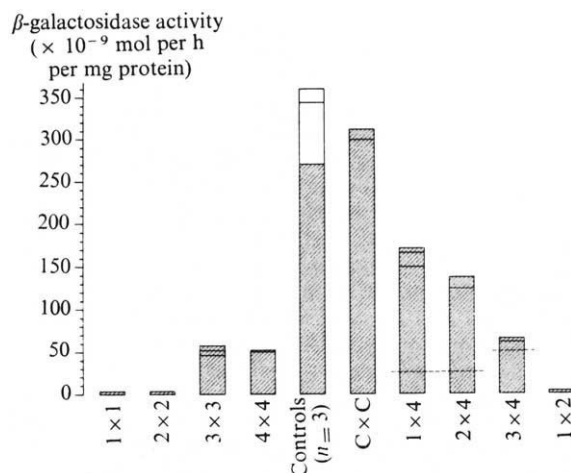


Fig. 1 β -Galactosidase assays on cell homogenates after fusion of cultured fibroblasts from patients with different variants of GM1-gangliosidosis. 2×10^6 cells from each strain were hybridised with inactivated Sendai virus¹³ under such conditions¹¹ that 50-80% of the nuclei were present in multinucleates. Two days after fusion cells were trypsinised, rinsed in saline and disrupted by sonication (2×30 s), and after protein analysis¹⁴ the β -galactosidase activity was assayed with 1 mM 4-methylumbelliferyl- β -D-galactopyranoside, pH 4.2, as described previously¹⁵. Independent experiments with the same cell strain; as controls three different normal strains were used; — — —, expected enzyme activity if no complementation occurs, that is the mean enzyme activity as a result of mixing equal numbers of both parental cells.

ganglioside (Table 1). Conditions for cell cultivation, fusion, collection and protein determination were identical to those described in Fig. 1. Whereas no enzyme activity was detected after type 1 $\times$ type 2 fusion a marked increase in GM1- β -galactosidase activity was observed after hybridisation of type 4 $\times$ type 1 cells. An additional fusion experiment of type 4 $\times$ type 1 cells was carried out with inactivated serum in the culture medium. This will inhibit proliferation of the unfused parental cells and thus the proportion of heterokaryons will be higher than if normal serum is used. This is reflected in the higher β -galactosidase activities in the last type 4 $\times$ type 1 fusion shown in Table 1.

These results showed that genetic complementation had occurred in the fusion between type 4 cells and type 1 or type 2 cells. Thus the adult form of GM1 gangliosidosis (type 4 variant) is caused by a different gene mutation than is present in the types 1 or 2 variants. Since there was no complementation after fusion of type 4 $\times$ type 3 cells these variants might be identical or determined by allelic mutations, as is the case for types 1 and 2 GM1-gangliosidosis¹⁷.

Although complementation could be detected by enzyme analyses in homogenates of heterokaryons it is not possible to evaluate the degree of restoration of activity since the proportions of nuclei from each parental cell present in the multi-

Table 1 β -Galactosidase assays using natural substrate after somatic cell hybridisation of different GM1-gangliosidosis variants

	Enzyme activity ($\times 10^{-9}$ mol per h per mg protein)	
	GM1- β -galactosidase	4-MU- β -galactosidase
Type 1 $\times$ type 1	3.5	1.8
Type 2 $\times$ type 2	4.3	4.0
Type 4 $\times$ type 4	10.5	13.4
Type 1 $\times$ type 2	<1	<1
Type 1 $\times$ type 4	44.1	38.6
Type 1 $\times$ type 4*	99.8	75.3
Control	193.8	246.7

*After fusion, cells were grown on medium with 10% foetal calf serum that had been treated with alkali (pH 10.7) for 3 h at 37 °C to destroy enzyme activity in the medium. Conditions of cell culture, hybridisation and collection were similar to those described earlier. GM1-galactoside- β -galactosidase activity was measured according to Ho *et al.*¹⁶.

nucleates are unknown. To solve this problem microchemical techniques were developed which facilitated quantitative enzyme assays in individual binuclear heterokaryons^{15,18}. Fibroblasts from the adult patient with type 4 GM1-gangliosidosis were hybridised with those from a type 1 variant under such conditions that about 8% of the hybrid cells are binucleate¹⁵. After fusion cells were grown for 24 h in a Falcon flask, they were then trypsinised and 2×10^4 – 4×10^4 cells were seeded in a dish with a thin plastic bottom. After 24–48 h of subsequent cultivation these dishes were frozen and freeze-dried immediately and small pieces of plastic each containing one binuclear cell or one non-fused mononucleate cell were dissected under microscopic control. β -Galactosidase assays in individual cells were carried out with synthetic substrate in submicrolitre volumes and fluorescence measurements were made in a microspectrofluorometer as described earlier^{15,18}.

The distribution of β -galactosidase activity in individual unfused control fibroblasts is shown in Fig. 2a. Results from three different cell strains were pooled, but also in cells from each cell strain there was a considerable variation of activity in the range 4×10^{-14} – 40×10^{-14} mol h⁻¹. In considering these data it should be realised that the enzyme activity is expressed per cell and that the total cellular dry mass and protein content in individual fibroblasts also show marked variations^{18,19}.

The mean β -galactosidase activity in control cell strains varied from 19×10^{-14} to 26×10^{-14} mol h⁻¹. Earlier experiments¹⁷ showed that the level of activity in parental fusions of control fibroblasts was approximately twice that of mononucleates. No β -galactosidase activity could be detected in

individual binuclear cells after parental fusions of type 1 cells while in parental fusions of type 4 cells mean activities of 3×10^{-14} and 5×10^{-14} mol h⁻¹ were found in two experiments; this is 10–20% of the activity in parental fusions of control fibroblasts (Fig. 2b).

After fusion of type 4 $\times$ type 1 cells in 21% of the binucleates no β -galactosidase activity could be detected; these probably represent fusions of type 1 $\times$ type 1 cells. In 27% of the binuclear cells the enzyme activity was similar to that in parental fusions of type 4 cells. But in 52% of the binuclear heterokaryons β -galactosidase activity was in the range 8×10^{-14} to 40×10^{-14} mol h⁻¹ (Fig. 2c). This is greater than in either of the parental cells before or after fusion and the range of activity is similar to that in mononuclear control fibroblasts. Thus genetic complementation after fusion of type 4 $\times$ type 1 cells results in restoration of β -galactosidase activity to normal control levels.

The mechanism responsible for the genetic complementation observed after fusion of type 4 cells with type 1 or type 2 cells is not yet understood. In human liver two forms of GM1-ganglioside- β -galactosidases (A and B) have been identified^{16,20} both of which are deficient in GM1-gangliosidosis²⁰. It has been proposed that the A form of β -galactosidase consists of a single polypeptide chain²¹ and that the B form is a multimeric aggregate of the A monomer⁹. On this basis the results of our hybridisation studies would indicate the occurrence of intragenic complementation. In a recent review, O'Brien⁹ does not exclude the possibility that the B form of β -galactosidase also contains amino acid sequences which are not common to the A form. In that case the complementation in type 4 $\times$ type 1 or 2 heterokaryons could still be intergenic. A third possibility is that the gene mutation in type 4 GM1-gangliosidosis involves a regulator whereas the mutations in type 1 and type 2 are structural mutations. The latter is supported by immunological studies^{9,22} which revealed the presence of cross-reactive material in cells from all patients tested with type 1 and type 2 GM1-gangliosidosis. Preliminary analyses of the deficient β -galactosidase in type 4 cells and cocultivation studies are suggestive for a regulator mutation in the adult type 4 GM1-gangliosidosis. Further investigations on the immunological and biochemical properties of the deficient enzyme and of the restored enzyme activity after genetic complementation are required to prove this hypothesis.

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H. GALJAARD
A. HOOGEVEEN
W. KEIJZER
H. A. DE WIT-VERBEEK
A. J. J. REUSER

Department of Cell Biology and Genetics,
Faculty of Medicine,
Erasmus University,
Rotterdam, The Netherlands

MAE WAN HO
D. ROBINSON

Department of Biochemistry,
Queen Elizabeth College,
University of London, UK

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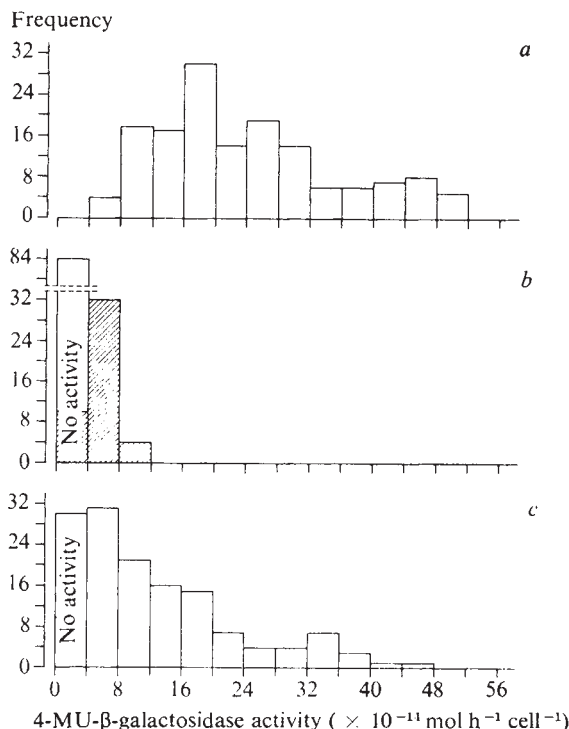


Fig. 2 β -Galactosidase assays in individual heterokaryons after fusion of type 4 $\times$ type 1 variants of GM1-gangliosidosis. β -Galactosidase activity was measured by incubating individual cells in 0.2 μ l 1 mM 4-methylumbelliferyl- β -D-galactopyranoside in 0.1 M-acetate buffer (pH 4.2) containing 0.02% (w/v) bovine albumin. Incubation was carried out during 2 h at 37 °C under paraffin oil using Lowry's "oil well technique"^{15,18}; after addition of 1 μ l 0.5 M carbonate buffer (pH 10.7) the fluorescence was measured in 1-mm glass capillaries with a Leitz microspectrofluorometer^{15,18} (objective 2.5 pl). *a*, Distribution of enzyme activities in non fused mononuclear control fibroblasts as measured in three different cell strains; *b*, enzyme activities in individual binuclear cells after parental fusion of type 1 $\times$ 1 (open columns) and type 4 $\times$ 4 (hatched columns) cells each measured in two independent experiments; *c*, enzyme activities in binuclear heterokaryons after fusion of type 4 $\times$ 1 cells as measured in three independent experiments.

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Influence of sexual dimorphism on foetal and placental weights in the rat

THE male is heavier than the female in the adults of most mammalian species. This trend is apparent at birth in humans¹ and in many other mammals including the sheep, goat, cow, rat and monkey². Little is known however, of the developmental basis for these birth weight differences. We have found that, one day before birth, male rat foetuses are 5% heavier than female foetuses, yet there seems to be no difference in the weights of their placentae.

Heavier foetuses are generally associated with heavier placentae³. This could be a causal relationship, where foetal growth is limited by placental function, as reflected by placental weight, or it may merely be a consequence of the common origin of the foetus and the foetal component of the placenta³. Whatever the reason, it might be expected that male foetuses would have heavier placentae than female foetuses. Here we examine the influence of sex on foetal and placental weights in the rat as part of a general study on the control of placental development and function. We have also tested the hypotheses that the sex of a conceptus has a general systemic effect on the growth of all other conceptuses in the litter and a local effect on its immediate neighbours since there is some evidence of similar effects in man⁴.

Thirty-four albino rats with a mean weight at mating of 238 ± 3 g (mean $\pm$ s.e.) were used. The day spermatozoa were found in a vaginal smear was called day 1 of gestation. On day 22, the day before expected parturition, each rat was killed with an overdose of sodium pentobarbitone, its uterus removed and the number and position of all implantation sites recorded. The uterus was then opened and each conceptus examined in detail. Only 'live' foetuses, which moved in response to pressure, were included in the results. The sex of each foetus was determined by the distance between the anus and the genital tubercle (about 2 mm in males and 1 mm in females). The internal genitalia were examined in 25 of the foetuses and were always found to agree with the external morphology. The foetal membranes and umbilical cord were removed from each conceptus; the foetus and placenta were wiped dry and weighed separately.

There were 9.9 ± 0.6 live foetuses per litter: 49.7% were males. Thirty-four conceptuses, about one per litter, died at various stages before day 22. The mean foetal weight per litter was 4.598 ± 0.056 g, the mean placental weight was 0.463 ± 0.006 g and the mean foetal weight : placental weight ratio was 10.049 ± 0.220 .

In the rat, conceptuses in the middle of both uterine horns tend to be heavier than those at either end⁵. Any selective positioning of male and female conceptuses along the horn

could have caused this trend or alternatively, have affected any appraisal of differences in male and female weights. No selective positioning was apparent however, since the numbers of males and females were respectively 27 and 26 at the ovarian end, 20 and 30 at the middle and 20 and 29 at the vaginal end of the horns. Horns containing foetuses of one sex only were excluded.

Sex differences in conceptus weights were determined in each horn to avoid between rat and between horn variation. Male foetuses were found to be 5.4% or 0.247 ± 0.030 g heavier ($P < 0.001$, $n = 55$) than female foetuses. The placentae of male foetuses were however, only 1.3% or 0.006 ± 0.008 g heavier (not significant). The foetal weight : placental weight ratio was thus higher (5.2%, $P < 0.005$) in males than in females.

The systemic effect of the number of males and the number of females in the litter on mean foetal and placental weights was examined by analysis of covariance. Larger litters, irrespective of sex, had smaller placentae $r = -0.528$ ($P < 0.01$, $n = 34$). At equal litter sizes however, there was no apparent difference in the effect of the number of males or the number of females on mean placental or foetal weights of either sex.

The local effect on each foetus and placenta of the sex of its immediate neighbours was assessed by classifying each foetus as having either 0, 1 or 2 neighbours of the opposite sex and then comparing foetal and placental weights between these classes. Comparisons were made within each horn to avoid between horn variation, consequently the number of horns with examples of two or more of these classes was reduced. Conceptuses were excluded if their immediate neighbour was dead, since the dead conceptus could not be sexed and may, itself, have had a local effect.

Male conceptuses seemed to be little influenced by the sex of their neighbour although male foetuses and placentae with two female neighbours were 3.8% and 4.5% heavier (not significant, $n = 7$) than those without female neighbours. No other male class comparisons yielded differences of greater than 0.7%. There was more indication that female conceptuses were influenced by the sex of their neighbour. Female foetuses with one male neighbour were 2.9% heavier ($P < 0.05$, $n = 27$) than those without male neighbours. Placentae of female foetuses with two male neighbours were 6.5% ($P < 0.05$, $n = 9$) and 8.6% (not significant, $n = 4$) lighter than those with one or no male neighbours respectively. No other female class comparisons yielded differences of greater than 1.3%.

The principal finding to emerge from the above results was that male foetuses are about 5.4% heavier than females but their placental weights are remarkably uniform. Similar observations have been reported in the monkey³. These findings raise a number of questions about the developmental basis of sexual dimorphism.

Sexual differences in growth rates of conceptuses, either by cellular enlargement or by multiplication, can be attributed directly to a genotypic difference and indirectly to various environmental differences evoked by the genotype. It has been shown that even at the blastocyst stage, the genotype of the conceptus can affect its own growth rate⁶. In this way the XX or XY chromosome complement may have a differential effect on the growth rate of cells, irrespective of environmental influences. While there is no evidence as yet for such a genetic effect, male-female weight differences seem to depend on more than just hypothalamic or gonadal hormones since weight differences are evident even in anencephalic foetuses⁷. The latter would be expected to have low, if any, levels of these hormones, at least in the second half of gestation⁸. On the other hand, if the sex chromosome complement intrinsically affects cell growth or division in the foetus, it is difficult to explain why it did not affect the placenta, an organ largely composed of foetal tissue.

Environmental factors which might influence male-female weight differences include the interaction of the foetus with its mother and gonadal hormones. The degree of antigenic interaction of mother and foetus in mice can influence placental and subsequently foetal size⁹. Since male foetuses possess the

greater antigenic dissimilarity they might be expected to have heavier placentae⁴. Our placental weight findings would however, seem to discount this hypothesis. Gonadal hormones are often used to explain sexual dimorphism. They do affect the differentiation of the reproductive tract of the foetus, so they might also affect its growth rate. The evidence of the anencephalic children however, tends to oppose this idea. In addition, if male gonadal hormones, generally considered to be anabolic, affect foetal growth, why do they not affect placental growth? Perhaps the placenta is not a target for gonadal hormones or, if it is, it may respond by improved efficiency rather than by weight. Differences in the efficiency of the placenta or of the foetus to use its placenta could mask any association of foetal and placental weights expected if foetal growth is indeed limited by placental function at the level of sexual dimorphism.

The possibility that each foetus, depending on its sex, releases or evokes circulating substances in its mother, that in turn affect the growth rates of other foetuses in the litter, was not supported by any evidence of systemic effects. On the other hand, it did seem that the sex of a foetus can influence the growth rates of its neighbours, presumably by releasing or evoking locally acting substances. Although this finding requires confirmation, there is good evidence that other hormones, notably prostaglandins, are produced in the uterus and have powerful local effects extending even to the ovary¹⁰.

In the rat and several other mammals, sexual dimorphism in relation to size is apparent even before birth. A closer examination of this phenomenon and its ramifications may well lead to a better understanding of the control of prenatal growth.

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N. W. BRUCE
N. NORMAN

Department of Anatomy and Human Biology,
University of Western Australia,
Nedlands, 6009,
Western Australia

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Prolactin levels and duration of *postpartum* anoestrus in lactating ewes

THE length of the *postpartum* anoestrous period in sheep is related to the degree of mammary stimulation. If the ewe is milked by hand twice a day or 'dried off' immediately after parturition, the first oestrus occurs 30-40 d *postpartum* in the 'Préalpes du Sud' breed; if the ewe is nursing her lamb(s)—suckling 10-12 times per day—*postpartum* anoestrus lasts for 60-80 d (ref. 1). A similar phenomenon has been described in women; menses reappear within 10 weeks *postpartum* in 80% of women who are not breast feeding, but in only 10% of those who are². In both species suckling is accompanied by large discharges of oxytocin and prolactin; this reflex secretion of prolactin decreases as lactation progresses, and is almost nonexistent by the time of return of cyclical ovarian activity.

Amenorrhoea is also associated with high blood levels of

prolactin in women who are not breast feeding, and menses return after the prolactin levels have been lowered by hypophysectomy or administration of a prolactin-suppressing drug like 2-bromo- α -ergo-cryptine³.

These facts all suggest that prolactin has an antigonadal action^{4,5}; we have therefore studied ovarian and pituitary activity in lactating ewes after denervation of the mammary gland to inhibit the afferent arc of the suckling reflex. Denervation can be achieved by several means⁶. Bilateral section of dorsal pathways of the spinal cord at T₁₁, and bilateral sympathectomy and section of the perineal nerves results in a disappearance of the normal prolactin surge observed during milking or suckling. A similar effect is observed after the bilateral section of the afferent roots of lumbar nerves 1, 2, 3 and 4, lumbar sympathectomy, and section of the perineal nerves. Unilateral section of the afferent nerves from the mammary gland shows that the spinal reflex resulting in a prolactin surge after teat stimulation is ipsilateral (Fig. 1).

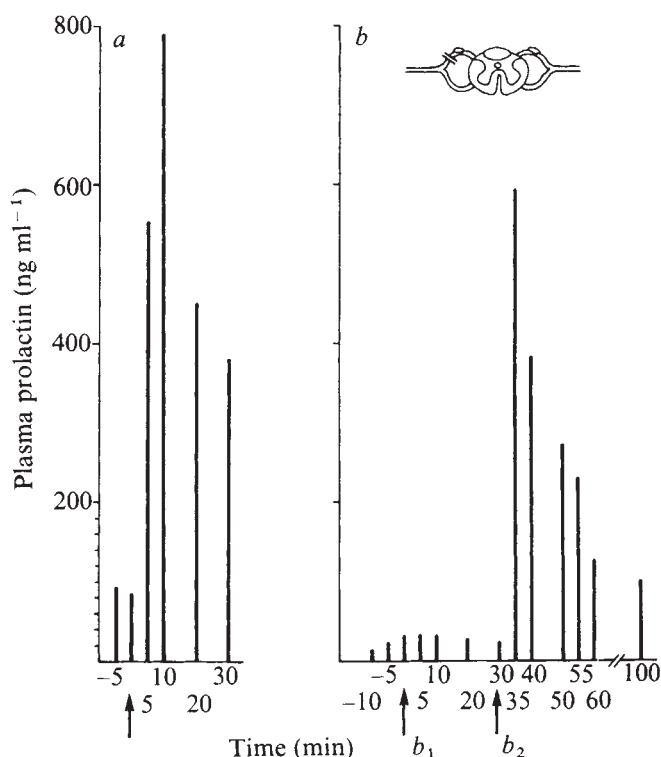


Fig. 1 Prolactin levels in suckled ewes before and after unilateral severance of lumbar nerves 1, 2, 3 and 4. Results expressed as ng ml⁻¹ (NIH, PS7). *a*, Reflex prolactin secretion triggered by suckling (arrow) in a normal ewe; *b*, after unilateral mammary denervation; *b*₁, disappearance of reflex prolactin secretion after ipsilateral teat stimulation (arrow); *b*₂, restoration of the reflex on contralateral teat stimulation (arrow).

All nervous connections between the mammary gland and the body were severed, including perivascular sympathectomy of the inguinal blood vessels, in five 'Préalpes du Sud' ewes during late pregnancy. After normal parturition, ewes were allowed to suckle their lambs, as were eight unoperated control ewes, which lambed at the same time. Blood samples were obtained twice daily for radioimmunoassay of prolactin and luteinising hormone (LH)^{7,8}, and oestrous behaviour was checked after blood sampling using a vasectomised ram. Laparoscopy was carried out on all the ewes between days 12-15, 25-31 and 40-50 *postpartum* to examine the state of the ovaries.

The normal *prepartum* surge of prolactin secretion and the

basal levels during lactation were unaffected in the denervated animals, but the suckling stimulus of the lambs was not followed by the normal reflex discharge of prolactin. As in previous experiments⁶, the growth of the lambs of these denervated ewes was normal.

In control animals, neither ovulation nor an LH peak was observed before 25 d *postpartum*; five of the eight animals exhibited silent ovulations preceded by an LH surge between days 25 and 50 *postpartum*, and only three of them came into oestrus during this period. In marked contrast, three of the five operated animals had ovulated without showing oestrus before day 14 *postpartum*, and all five animals had ovulated and shown oestrus before day 50.

These results establish that it is the suckling stimulus, and not lactation itself, that in some way suppresses the resumption of cyclical ovarian activity. In preliminary experiments we have been able to show that pharmacological suppression of prolactin secretion in suckling ewes with 2-bromo- α -ergocryptine (CB154, Sandoz) is also followed by an early restoration of cyclical ovarian activity⁹. Therefore it seems reasonable to conclude that it is the surges of prolactin secretion specifically induced by suckling that are in some way responsible for the suppression of ovarian activity during lactation.

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G. KANN
J. MARTINET

Laboratoire de Physiologie de la Lactation,
INRA, 78350-Jouy-en-Josas, France

Received June 10; accepted July 21, 1975.

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Structure of DNA molecules in yeast meiosis

THE nuclear DNA of the yeast *Saccharomyces cerevisiae* has been the subject of a detailed electron microscopic examination^{1,2}. These studies have revealed long, continuous molecules of lengths up to 355 μm , which correspond to the molecular weights of nuclear DNA estimated from sedimentation analysis in sucrose gradients. Several replication structures (bubbles) were observed along single molecules^{2,3}, as found in other eukaryotes^{4,5}. The most useful observations were made on selected replicating DNA³ and on DNA of mutants that were arrested just after the initiation of DNA synthesis⁶. We have started to study the structure of DNA molecules during meiosis because of its direct relevance to the process of recombination. Models to explain recombination (for review see ref. 7) are based mostly on genetic data from meiotic analysis, among which yeast tetrads occupy an important position. The various models have molecular implications which could best be tested by the direct examination and quantification of the interactions among DNA molecules, and between DNA and proteins, during meiosis.

Three unique features are expected to characterise meiotic DNA. First, the longer duration of the premeiotic DNA synthesis may be the result of the presence of fewer replicons than in the vegetative replication, as found in *Triturus*⁸. It has also been suggested that certain regions remain unreplicated during the premeiotic S period and are completed only during zygotene⁹. The second unique feature of meiotic DNA inter-

actions is pairing; the four double helix molecules that constitute the four chromatids of a bivalent (provided one accepts the unineme structure of the chromatid⁹) must be paired intimately. The third feature, breakage and reunion between the paired molecules, is expected from the genetic data to occur 5–15 times along an average-sized bivalent of yeast¹⁰. This is the act of recombination itself, based probably on base pairing between antipolar single-stranded molecules from different chromatids. Depending on the model⁷, single-stranded regions either remain in the backbone of the DNA molecules (chromatids), and are later repaired by polymerase and ligase, or they remain projected from the double-stranded molecules and are later digested by exonuclease/ligase repair activity.

Vegetative cells of strain 419/1 that were labelled with ³H-6-uracil as described in the legend to Fig. 1, were transferred to sporulation medium (SPM) to give meiosis with kinetics

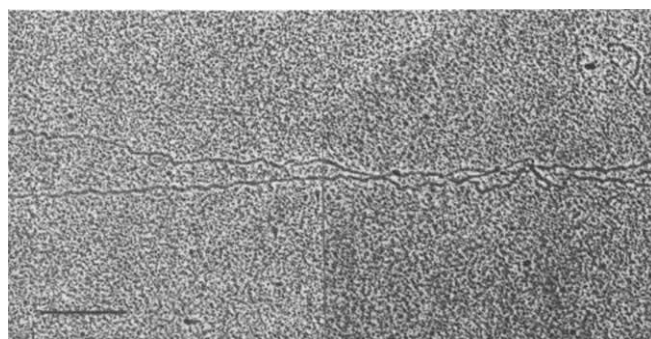


Fig. 1 Pairing between two DNA molecules. These molecules

were paired over a length of 30 μm and were clearly connected to

each other in at least two other places. The threads linking the

two molecules here are probably single-stranded, at least over

part of their length. DNA was prepared for electron microscopy

by a modified Kleinschmidt technique¹⁴. The diploid yeast strain

419/1 is a *ura1/ura1* homozygous derivative (obtained after low-

dose ultraviolet irradiation) of strain 419 (ref. 11). DNA was

prepared from meiotic cells for equilibrium sedimentation

analysis after they were grown overnight at 30 °C in PSP2

medium¹² supplemented with adenine (40 $\mu\text{g ml}^{-1}$), uracil

(20 $\mu\text{M ml}^{-1}$) and ³H-6-uracil (3 $\mu\text{Ci ml}^{-1}$, specific activity of

label 13.6 Ci mM⁻¹), to a titre of 10⁷ cells ml⁻¹ and were washed

and resuspended in SPM for 6.75 h. Spheroplasts were pre-

pared from 5 ml of the culture by 30-min incubation with

Glusulase (Endo Lab.) in spheroplasting buffer¹³, lysis was

obtained following their transfer to a lysis buffer¹³, and the lysate

was mixed with a saturated solution of CsCl, to a volume of 5 ml,

with an average buoyant density of 1.690 g ml⁻¹. Density gradients

were obtained in cellulose nitrate tubes following 60-h runs in

the Spinco type 50 rotor at 35,000 r.p.m. The tubes were pierced

and dripped into 35–40 tubes, from which samples were counted

for radioactive label, following an overnight alkali treatment

(NaOH 0.5 N) and TCA precipitation (BSA added as carrier).

The nuclear DNA was separated from the mitochondrial DNA

by about 12 fractions, the former giving a very sharp peak of 2–4

fractions, from which DNA was spread for the electron micro-

scopy. Samples (10 μl) were taken from the nuclear peak fractions

of the CsCl gradients. The DNA concentration in these fractions

was estimated to be about 1–2 $\mu\text{g ml}^{-1}$. Each sample was added

to 30 μl of a freshly prepared mixture, containing 0.1 mg ml⁻¹

cytochrome c, 3 M ammonium acetate buffer (pH 6.8) and 50%

formamide. After gentle agitation, 5 μl were withdrawn and

spread, in a wax-coated depression plate, on a 1-ml hypophase

containing 0.3 M ammonium acetate buffer (pH 6.8) and 10%

formamide. A carbon-coated grid was used to pick up a small

drop from the hypophase surface. The grid was then dehydrated

in absolute ethyl alcohol and shadowed with platinum–palladium

(80:20) at an angle of 10° in vacuum of 5 $\times 10^{-5}$ mmHg. A

JEOL-JEE 4B high vacuum evaporator with a rotatory table

(60 r.p.m.) was used for the shadowing. A JEOL 7A electron

microscope was used, with 40- μm foil objective aperture and

80 kV accelerating voltage. Magnification was calibrated with

a carbon grating replica, 28,800 lines per inch (Ernest F. Fullam,

Inc., Schenectady, New York). DNA control standards were

Herpes simplex type I DNA (mean length 54.3 $\pm$ 0.3 μm) and

ϕ X174 RF DNA (mean length of 1.8 $\pm$ 0.3 μm) that were received

from Y. Shlomai and A. Razin, respectively. Bar represents

0.5 μm .

slightly faster than in related strains^{11,12}: the premeiotic DNA synthesis took place between 4 and 7 h, the first meiotic division took place at about 8 h, recombination commitment (ADE colonies) was from 5 to 8 h and haploidisation was from 7 to 9 h. The short duration of each meiotic stage and the asynchrony in the culture (estimated to be 2–3 h) resulted in a notable degree of overlapping between the premeiotic replication and the early meiotic stages.

Vegetative DNA spreads, prepared from the nuclear peaks of CsCl gradients, appeared in the electron microscope to be similar in every respect to the molecules described by Petes *et al.*^{1–3}. Linear molecules were of various lengths up to 300–400 μm . A few molecules were in replication, with 'bubbles' ('eye-forms') of various lengths. We also observed small circular molecules, 2 μm in circumference, as described previously¹⁵. Some of these small molecules were supercoiled and a few were replicating, showing a closed, symmetrical, bacterial-like replicon.

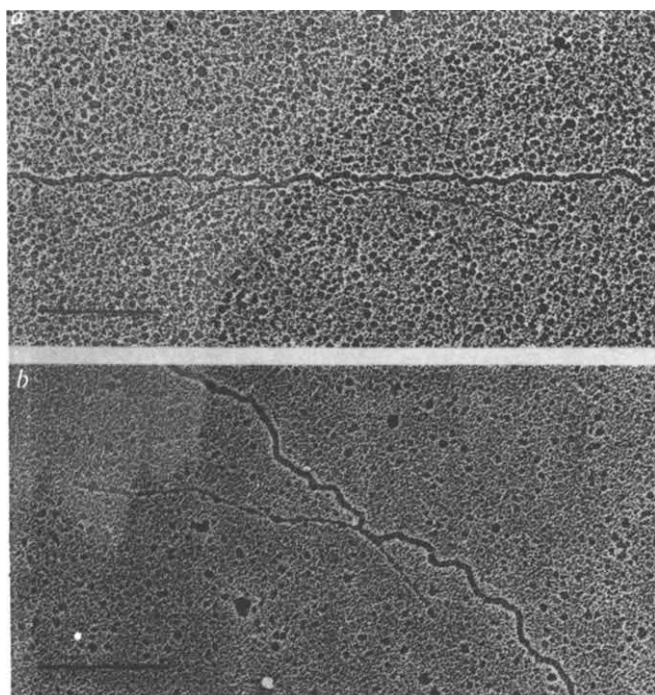


Fig. 2 Parts of linear double-stranded molecules with two single-stranded projections each. *a*, The lengths of the projections are 1.0 and 0.8 μm and the intermediate region (between the branching off points of the projections) is 0.2 μm ; *b*, the lengths of the projections are 1.16 and 0.28 μm and the intermediate region is 0.1 μm . Bars represent 0.5 μm .

Meiotic DNA spreads were prepared from samples taken at 5, 6, 6.75, 7.5 and 8 h in sporulation. At the two earliest times, we observed 'bubbles' of various sizes, including some much longer than those found in vegetative DNA. These molecules could, however, be paired molecules that were merged over short distances. As yet we find it difficult to resolve with certainty very long 'bubbles' from paired molecules. Paired molecules were also common in our preparations from 6 h onwards, including pairing arrangements of three and four molecules (see, for example, Fig. 1). Comparable pairing configurations were not observed in any of our vegetative DNA preparations.

The most characteristic feature of the meiotic DNA molecules were single-stranded projections observed quite commonly in the 6-h preparation and with higher frequency in the 6.75-h preparation. The projections usually occur in pairs (Fig. 2) and

sometimes they appear repeatedly along the same molecule. In most cases, the single-stranded projections seem quite convincingly to be an integral part of the main double-stranded molecule. We did not see such single-stranded projections in the vegetative DNA preparations, nor did we see them on the 2- μm circles in the meiotic DNA preparations. (The circles in the vegetative and meiotic preparations had a similar appearance.) In some preparations there seemed to be a certain affinity between the single-stranded projections of different molecules, resulting in complex configurations. At this preliminary stage, we cannot resolve these, or other complex molecules.

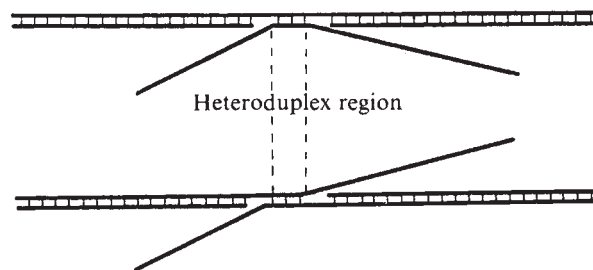


Fig. 3 Two possible interpretations of the molecule in Fig. 2*a*.

The pairs of single-stranded projections are most easily interpreted as the simplest recombination, or gene conversion, configurations outlined in Fig. 3. We believe these projections to be the result of genetic recombination, but we cannot exclude the possibility that they are intermediate structures towards the act of recombination. If, however, the former interpretation is correct, and Fig. 3 is taken at face value, the length of the heteroduplex region that resides between the two projections can be estimated. In Fig. 2*a*, this length is approximately 0.2 μm and in other pairs of projections the length of the intermediate region was found to be between 0.1 and 0.5 μm . The heteroduplex region in Fig. 2*a* is about 600 nucleotides long, and this corresponds well with the length estimated from genetic data on coconversion in yeast¹⁶.

Electron microscopic analysis of the structure of meiotic DNA can aid the understanding of genetic recombination at the molecular level and the choice between the various proposed models⁷. Our experience with this material, however, has convinced us that a systematic study of the variety of configurations encountered can only be made if accompanied by the use of mutants or treatments that accumulate intermediate configurations. As no true *rec⁻* mutants are available in yeast, we are now studying DNA structure in the *cde* mutants¹⁷, which block meiosis¹¹ at defined stages of DNA synthesis or nuclear division.

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GIORA SIMCHEN
ADAM FRIEDMANN

Department of Genetics,
The Hebrew University,
Jerusalem, Israel

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Major mRNA species from spinach chloroplasts do not contain poly(A)

POLY(A) is a constituent of most animal cytoplasmic mRNAs^{1,2} and has been demonstrated in rapidly-labelled, polysomal RNA in plants^{3,4}. Verma *et al.*⁵ have shown that cellulase mRNA, isolated from auxin-treated pea epicotyls, contains poly(A). Although a significant proportion of eukaryotic polysomal mRNAs are known to lack poly(A)^{6–8}, the presence of poly(A) is still regarded as a characteristic feature of unfractionated preparations of eukaryotic mRNA. By contrast, poly(A) has not been detected in mRNA from prokaryotic cells⁸. Several reports^{9–11} have indicated that poly(A) is present in HeLa cell mitochondrial RNA, although Groot *et al.*¹² were unable to detect poly(A) in yeast mitochondrial RNA. Here we describe experiments in which we have assayed for the presence of poly(A) in spinach chloroplast RNA by two methods; (1) hybridisation of chloroplast RNA with ³H-poly(U) and (2) binding of chloroplast RNA, pulse-labelled *in vivo*, to oligo(dT) cellulose. Additionally, the mRNA activities of chloroplast poly(A)-containing RNA and non-poly(A)-containing RNA (hereafter referred to as poly(A)⁺ RNA and poly(A)[−] RNA respectively) have been investigated by measuring their translation into specific polypeptides in cell-free extracts from *Escherichia coli*. From the results, we conclude that chloroplast mRNA lacks poly(A).

isolated from leaves labelled *in vivo* for 3 h with ³²P-orthophosphate¹⁴. For comparison of poly(A)⁺ RNA content, leaf total RNA was prepared from the same tissue. We have previously shown¹⁴ that in leaves pulse-labelled with ³²P-orthophosphate for 3 h, label is incorporated into mature rRNA, rRNA precursors and 'polydisperse' RNA in both chloroplast and leaf total RNA preparations. The poly(A)⁺ RNA content of the samples was determined by fractionation on columns of oligo(dT) cellulose as described by Clegg and Kennedy¹⁵. The results (Table 2) show that whereas 10.5% of the leaf total labelled RNA is retained by oligo(dT) cellulose, and is subsequently eluted in low salt buffer, only 2.1% of the RNA extracted from the crude chloroplast preparation is thus retained by oligo(dT) cellulose. A significant proportion of the poly(A)⁺ RNA extracted from the crude chloroplast preparation probably represents cytoplasmic contamination, since washing the chloroplasts once with isotonic medium before nucleic acid extraction reduces the poly(A)⁺ RNA content to about 1%; washing the chloroplasts a second time does not further reduce the poly(A)⁺ RNA content. Polyacrylamide gel electrophoresis of the ³²P-labelled poly(A)⁺ RNA fraction in leaf total RNA showed it to be heterogeneous in size, ranging from 0.2 × 10⁶ to 4 × 10⁶ molecular weight (not shown). In contrast, the poly(A)⁺ RNA from washed chloroplasts was much smaller in size, the majority migrating ahead of 4S RNA. This suggests that the small amount of poly(A)⁺ RNA in the washed chloroplast preparation does not simply arise from cytoplasmic contamination.

To establish whether or not it is the small amount of chloroplast poly(A)⁺ RNA, or the poly(A)[−] RNA which possesses mRNA activity, the two fractions were assayed for their ability to direct the synthesis of specific polypeptides in a cell-free protein synthesising system from *E. coli*. The products of cell-free protein synthesis were analysed by polyacrylamide gel

Table 1 Determination of poly(A) content by hybridisation with ³H-poly(U)

Source of RNA	Amount (μg)	RNase-resistant c.p.m.	c.p.m. minus no RNA control	Poly(U) in hybrid (μg)	% poly(A)
No RNA	—	87	—	—	—
<i>E. coli</i> ribosomes	50	78	—	—	ND
	100	83	—	—	ND
Bacteriophage MS2	50	350	263	0.0018	0.0034
	100	608	521	0.0036	0.0034
Spinach leaf (total)	50	6 153	6,066	0.043	0.080
	100	11,362	11,275	0.079	0.074
Spinach chloroplasts	50	330	243	0.0017	0.0032
	100	621	534	0.0037	0.0035

Hybridisation mixtures contained the components of the standard assay described by Gillespie *et al.*¹³ 50 or 100 μg of the non-radioactive RNA shown and 1 μg of ³H-poly(U) (specific activity 143 × 10³ c.p.m. μg^{−1}; prepared as described by Eaton and Hutchinson¹⁶) in a total volume of 125 μl. After incubation at 36 °C for 24 h, 2.5 ml of 0.5 M NaCl, 10 mM MgCl₂, 10 mM Tris-HCl (pH 7.2) was added, followed by 15 μg RNase A and 5 μg DNase I, and incubation continued at 30 °C for 1 h. TCA-insoluble radioactivity was determined as described by Gillespie *et al.*¹³. The poly(A) content of the RNAs was calculated from the equation: poly(A) content = (μg hybrid poly(U) × 0.933)/(μg RNA) × 100. Spinach leaf total RNA, chloroplast RNA and bacteriophage MS2 RNA were prepared as described previously^{14,15}. *E. coli* rRNA was described by Avery¹⁷.

ND, Not detectable.

Samples of spinach chloroplast RNA were assayed for their poly(A) content by hybridisation with excess ³H-poly(U) in conditions reported to be optimal for poly(A)-poly(U) hybrid formation¹⁸. For comparison, samples of leaf total RNA (presumably containing poly(A) tracts), *E. coli* rRNA and bacteriophage MS2 RNA (the latter two lacking poly(A) tracts¹³) were similarly analysed. The results (Table 1) show that the poly(A) content of chloroplast RNA (0.003%) is some 25 times less than that of leaf total RNA, and is nearly identical to that of MS2 RNA (Table 1).

To investigate the poly(A)⁺ RNA content of pulse-labelled chloroplast RNA, RNA was extracted from chloroplasts

electrophoresis. The gel profiles obtained (Fig. 1) show that the product synthesised in the presence of chloroplast poly(A)[−] RNA (Fig. 1a) contains two discrete radioactive polypeptides of molecular weights 52,000 and 35,000. We have reported previously a very similar pattern of incorporation directed by unfractionated spinach chloroplast RNA, and established the identity of 52,000 molecular weight product as being the large subunit of Fraction I protein¹⁵. The identity of the 35,000 molecular weight product is at present unknown. The product synthesised in the presence of poly(A)⁺ chloroplast RNA (containing additionally 100 μg *E. coli* rRNA as carrier) contains no discrete, high molecular weight polypeptides

(Fig. 1b) and is qualitatively and quantitatively similar to the product synthesised in the presence of 100 μ g *E. coli* rRNA alone (Fig. 1c). From these results we conclude that the mRNAs for the large subunit of Fraction I protein and the 35,000 molecular weight polypeptide lack poly(A) tracts. This does not entirely exclude the possibility that other chloroplast mRNAs may contain poly(A), since they could be translated with a low efficiency in the *E. coli* cell-free system. This possibility is unlikely, however, since the products of incorporation directed by poly(A)⁻ RNA are qualitatively similar to those synthesised in intact, isolated chloroplasts¹⁵.

The most likely explanation of the results described here is that spinach chloroplast mRNA lacks poly(A); tracts of less than about 20 adenylate residues would not have been detected². In view of the complete lack of knowledge surrounding the

Table 2 Fractionation of pulse-labelled RNA on oligo(dT) cellulose columns

Source of ³² P-labelled RNA	Radioactivity applied to oligo(dT) cellulose column (c.p.m.)	Radioactivity eluted in low salt fraction (c.p.m.)	Poly(A) ⁺ RNA content (%)
Leaf (total)	50 × 10 ⁴	52,676	10.5
Crude chloroplasts	25 × 10 ⁴	5,251	2.10
Chloroplasts washed × 1	25 × 10 ⁴	2,623	1.05
Chloroplasts washed × 2	25 × 10 ⁴	2,717	1.09
<i>E. coli</i> ribosomes	100 × 10 ⁴	212	0.021

Leaves (5 g) excised from 15-d-old spinach plants were labelled for 3 h with ³²P-orthophosphate by way of their petioles¹⁴. Leaf total nucleic acid and crude chloroplasts were prepared¹⁴; for washed chloroplasts, the crude chloroplast pellet was resuspended in 5 ml of 0.35 M sucrose, 2 mM EDTA, 2 mM sodium isoascorbate and repelleted by centrifugation at 2,500g for 2 min. Chloroplast nucleic acid was prepared by the phenol-detergent method¹⁴. Ethanol-precipitated nucleic acid samples were dissolved at a concentration of 1 mg ml⁻¹ in 50 mM MES-NaOH buffer (pH 7.0), 2 mM MgCl₂ containing 5 μ g ml⁻¹ DNase I (RNase-free) and the samples incubated on ice for 20 min. Sodium dodecyl sulphate was then added to 0.5% and low molecular weight ³²P-labelled contaminants and DNase digestion products removed by exclusion chromatography on Sephadex G-25 columns. RNA samples were separated into poly(A)⁺ and poly(A)⁻ fractions on temperature-controlled oligo(dT)-cellulose columns as described by Clegg and Kennedy¹⁸. Poly(A)⁺ RNA was eluted from oligo(dT) cellulose with low salt buffer (10 mM Tris-HCl (pH 7.5), 0.1% SDS). The recovery of the radioactivity applied varied from 98% to 105%. ³²P-labelled *E. coli* rRNA was prepared as described by Avery¹⁷. Approximate specific activities of RNAs; leaf total, 4.1 × 10³ c.p.m. μ g⁻¹; chloroplast 2.3 × 10³ c.p.m. μ g⁻¹; *E. coli*, 34 × 10³ c.p.m. μ g⁻¹.

function of poly(A) in eukaryotic cytoplasmic mRNAs, no general significance can be placed on this finding at present, but it does reinforce the conclusion that poly(A) is not essential for messenger function^{19,20}. If the lack of poly(A) in chloroplast mRNAs proves to be a general phenomenon, it will be another respect in which chloroplasts resemble prokaryotes.

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A. M. WHEELER
M. R. HARTLEY

Biological Sciences,
University of Warwick,
Coventry CV4 7AL, UK

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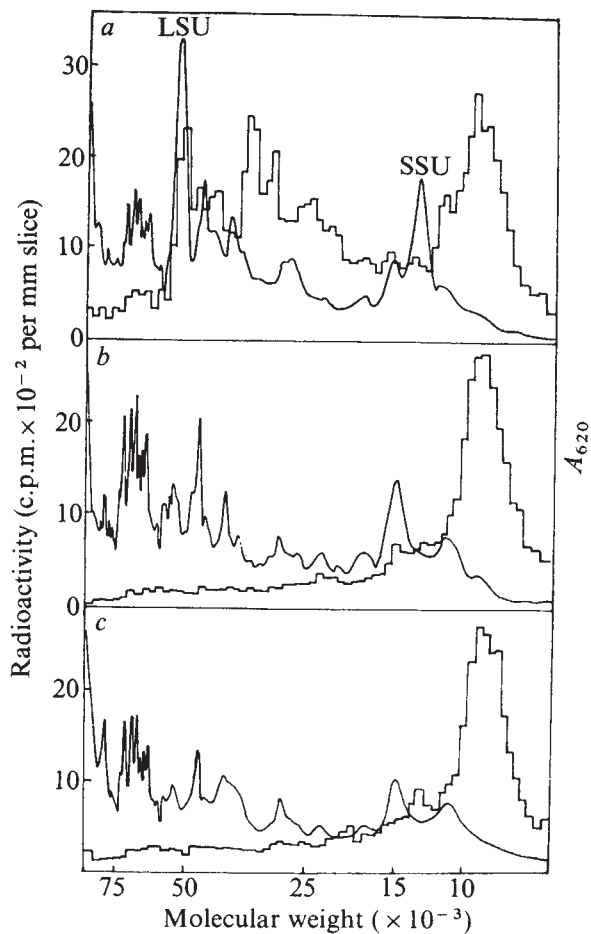


Fig. 1 Gel electrophoresis of the products of chloroplast poly(A)⁺ and poly(A)⁻ RNA-directed protein synthesis in cell-free extracts from *E. coli*. Chloroplast RNA was prepared as previously described¹⁴ and 0.5 mg aliquots separated into poly(A)⁺ and poly(A)⁻ RNA fractions on oligo(dT)-cellulose columns¹⁸. The poly(A)⁻ RNA, which passed through the column in 100 mM LiCl, 1 mM EDTA, 0.1% SDS, 50 mM Tris-HCl (pH 7.5) was ethanol precipitated directly from the eluant. The poly(A)⁺ RNA, eluted in low salt buffer (see legend of Table 2) was made 100 mM in NaCl; 100 μ g *E. coli* rRNA was then added as carrier before ethanol precipitation. A sample containing 100 μ g *E. coli* rRNA was similarly treated to serve as a control. These RNA samples were assayed for their ability to direct protein synthesis in *E. coli* S-30 extracts by methods previously described¹⁵. Products were analysed by SDS-polyacrylamide gel electrophoresis¹⁵. a, Incubation mix contained poly(A)⁻ RNA; b, incubation mix contained poly(A)⁺ RNA; c, incubation mix contained 100 μ g *E. coli* rRNA. —, Absorbance at 620 nm; histogram, radioactivity. LSU and SSU refer to the large and small subunits respectively of Fraction I protein added to sample (a) as marker.

Auxin, carbon dioxide and hydrogen ions

HYDROGEN ions can partially mimic the effect of indoleacetic acid (IAA) on plant cell elongation¹. This observation has been elaborated in a proposal that the initial action of IAA causes the activation of a proton pump at the cell membrane. The pumping of protons would cause a lowering of the pH at the cell wall, leading to wall loosening². This proposal has been supported in studies in which the pH of the medium surrounding oat coleoptile³⁻⁵ or etiolated pea stem⁶ sections was observed to drop when auxin was applied. This acidification was most pronounced when tissue sections were used from which the epidermis with the cuticle had been peeled off³, and was presumed to be caused by the proton pump activated by IAA.

An increase in cell respiration occurs when tissues respond to exogenous IAA (ref. 7). The IAA-induced pH drop observed in the solutions surrounding peeled tissue sections may be attributable to increased leakage of CO₂ from the tissue and subsequent formation of carbonic acid. To investigate this possibility, we measured CO₂ concentrations in the solutions surrounding pea epicotyl sections challenged with IAA.

Pea seeds (*Pisum sativum* L. cv. Alaska, lot 401 from Burpee Seed Co., Riverside, California) were germinated in the dark at room temperature (21 °C). Subapical 10-mm sections from the third internode were cut from 8-d-old seedlings. In peeled section experiments, the epidermis with cuticle was removed with fine forceps³. After the experimental period, the section lengths were measured, the pH of the medium taken, and the CO₂ contents of the solutions were measured with a Natelson model 600 microgasometer (Scientific Industries, Springfield, Massachusetts), which manometrically measures CO₂ in solutions.

Table 1 Effect of IAA on pH and CO₂ in the solution surrounding etiolated pea stem sections*

Conditions	pH ± s.d.	mM CO ₂ ± s.d.	Calculated pH
Unpeeled	6.04 ± 0.09	1.6 ± 0.09	6.02
Unpeeled + IAA	5.81 ± 0.10	3.8 ± 0.06	5.73
Peeled	5.87 ± 0.08	1.9 ± 0.09	5.97
Peeled + IAA	5.45 ± 0.11	5.3 ± 0.56	5.45
No sections	6.30 ± 0.00	0.5 ± 0.12	6.15

*Prepared sections were stirred in 200–300 ml 1.0 mM KH₂PO₄ buffer, pH 6.25 for 150–200 min. Rinsed sections were divided into lots of 45 and placed in 50 ml beakers containing 5 ml 1.0 mM KH₂PO₄ buffer, pH 6.30. After 30 min, the initial pH reading was taken and IAA was added to 10 μM. Beakers were sealed with Parafilm and allowed to stir for 3 h at which time readings were taken³.

Measurements of pH showed that IAA induced some acidification in the solution surrounding unpeeled sections, but that the acidification was more pronounced when peeled sections were used (Table 1). These data confirm previous results reported for unpeeled⁶ and peeled³ sections. The CO₂ measurements showed that when there was a drop in pH, there was also a rise in the CO₂ content of the solution. Using a K_a for total CO₂ in solution of 4.2×10^{-7} and a pK_a for the buffer used of 7.2 we were able to calculate the pH of the solution expected if only CO₂ was involved in the acidification⁸. These calculated pH values (Table 1) are quite similar to the measured pH, indicating that dissolved CO₂ was probably responsible for the IAA-induced pH reduction.

We carried out a time-course experiment to further examine the correlation between dissolved CO₂ and reduction in pH. When peeled sections were exposed for varying times to IAA, the results confirmed the correlation between increases in CO₂ and decreases in pH in the surrounding medium. Once again,

Table 2 Time course of pH and CO₂ changes in solution surrounding IAA-treated etiolated pea stem sections

Hours in IAA	pH	mM CO ₂	Calculated pH
0	6.1	0.5	6.15
1	5.8	3.4	5.73
2	5.6	4.4	5.63
3	5.3	5.8	5.43

the CO₂ effect was calculated to be adequate to account for the pH drop observed (Table 2).

To determine the relationship between the two correlated phenomena in solution, we carried out experiments in flasks with KOH-soaked filter paper in a centre well with the sections and solution surrounding the well. As the KOH would neutralise any CO₂-caused acidification, any pH reduction would then be caused by other phenomena. That little pH reduction was observed in such an experiment (Table 3) suggests a cause and effect relationship between increased CO₂ and reduction of pH during IAA treatment. It has been shown that exogenous CO₂ increases can induce cell elongation⁹. In all of our experiments, cell elongation (approximately 2 mm in 3 h) occurred only in IAA-treated tissue.

Table 3 Effect of KOH-soaked paper in a centre well on pH and CO₂ in solution surrounding etiolated pea stem sections*

Conditions	pH ± s.d.	mM CO ₂ ± s.d.	Calculated pH
Unpeeled	6.15	1.2	6.06
Unpeeled + IAA	6.11 ± 0.09	1.1 ± 0.08	6.07
Peeled	6.15	1.4	6.04
Peeled + IAA	6.09 ± 0.10	1.3 ± 0.09	6.04

*Sections were treated as described in Table 1, except that the experiment was run in a flask with a centre well, containing filter paper soaked with 10% KOH or water (control).

The source of the increased CO₂ in our experiments is probably increased cell respiration induced by IAA, the gas dissolving to form carbonic acid, which dissociates into bicarbonate and hydrogen ions. Plant cuticles are generally impervious to CO₂, and consequently the pH drop in the solution would be expected to be more pronounced in peeled than unpeeled section experiments¹⁰.

Our data tend to negate the hypothesis that the pH drop observed in the solutions surrounding IAA-treated tissue is attributable to a direct pumping of protons^{3,4}. It is possible that bicarbonate is a counter ion which accompanies hydrogen ion secretion. The data do not rule out the possibility that the primary event in cell elongation is indeed a lowering of the pH at the cell wall.

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MARK SLOANE
D. SADAVA

Joint Science Department,
The Claremont Colleges,
Claremont, California 91711

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matters arising

Interarc spreading in the Carpathian area

BOCCALETTI and GUAZZONE (ref. 1) have invoked the interarc spreading hypothesis to explain the genesis of the present structure of the Mediterranean area. We consider it necessary to clear up some fundamental points regarding one of the sectors they discussed.

The area inside the Carpathian Bend (including the Transylvania Basin in Rumania) was presented by Boccaletti and Guazzone as an interarc basin, the front and back inarc parts of which would be the Neogene volcanic zones in the East Carpathians and Apuseni Mountains. Because such a hypothesis has so far not been examined and argued but only stated²⁻⁴ we point out here the main geological facts which must be considered.

First, the basement of the Transylvanian Basin comprises both metamorphic and igneous (pre-Cenomanian) rocks, similar to those in the Carpathians⁵. Second, the sedimentary undeformed cover includes Palaeogene and early Miocene deposits continuous over large areas⁶. Third, there have so far been no observations indicating anomalous heat flow in this area.

The first point precludes any interpretation of the Transylvanian area as an interarc basin. If, however, this evidence were not considered, the second point would preclude a late Miocene age for the spreading process. Nevertheless, if the spreading were of that age, the Transylvanian Basin, being younger than the Pannonian Basin, should have a higher or comparable heat flow to that of the latter.

Finally, we emphasise that Karig⁷ developed the idea of interarc spreading for volcanic island arcs; his hypothesis cannot, however, be extended indiscriminately to apply to continental areas such as that inside the Carpathian Bend.

DAN P. RADULESCU

Mineralogical Department,
University of Bucharest

MIRCEA SANDULESCU

Institute for Geology and
Geophysics,
Bucharest, Romania

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⁷ Radulescu, D. P., and Sandulescu, M., *Tectonophysics*, 16, 155 (1973).

DRS BOCCALETTI AND GUAZZONE REPLY

—The structural model comprising a consuming (or contracting) zone, a folded arc, a magmatic arc, and spreading (or distended) zones, after Karig's formulation¹ and after additional ideas by Dickinson² and Dewey *et al.*^{3,4} must be considered the best model to date for investigating fossil continental margins, both in wide, contracting palaeo-oceans and in small, contracting palaeomarginal basins. Differences between oceanic and continental arcs are probably only a matter of differences in the ages of the contracting processes and differences in the dimensions of contracting areas. Consequently, different stages of maturity probably also occur in areas beyond the arcs.

In that sense all Mediterranean basins, both present and fossils, are very different from those of the western Pacific. Even the Tyrrhenian Basin, which is generally accepted as a typical marginal basin in the Mediterranean area (with active subduction, typical polarity and migration, and typical folded and magmatic arcs) also shows a crustal substratum that is strongly thinned and very hot. It seems to be complicated, however, by many sialic microfragments and sialic seamounts. These sialic 'lenses' are probably actual examples of a segmentation stage in the pre-Tyrrhenian crust. The original floor of the Tyrrhenian Sea, as well as the substrata of the other Mediterranean basins, was never entirely substituted by typical oceanic crust.

The points raised by Radulescu and Sandulescu⁵ can be explained as effects of a low level of maturity of evolution in the Carpathian arc-trench system, where the back-arc crust has been distended, thinned and in places fragmented to a certain degree. In fact, Tortonian sediments directly overlie the basement in many places of the Transylvanian Basin⁶. Some spreading may occur under cover, caused by discontinuous subduction; perhaps that can be

correlated with rotated arc migration. Incidentally, the age of the oldest deposits contemporaneous with the spreading in the basin must be related to later surface volcanism.

Stegena⁷⁻⁸ suggest that the inter-Carpathian area should be considered to be "warm". Comparisons between the Pannonian and Transylvanian basins could not give the same result if the directions of the investigation profiles are changed. In fact, it has been clearly demonstrated that the northern Carpathians are inactive whereas the south-eastern Carpathian Arc is still active⁹. Geothermic correlations between different basins should take into account the different thicknesses of their covers. The only conjecture that we can advance about the comparison between the Pannonian and Transylvanian basins is that the second is probably less 'mature' than the first.

*Instituto di Geologia dell'Università
di Firenze,
Italia*

¹ Karig, D. E., *J. geophys. Res.*, 76, 2542-2561 (1971).

² Dickinson, W. R., *Am. J. Sc.*, 272, 551-576 (1972).

³ Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, 76, 3179-3206 (1971).

⁴ Dewey, J. F., Pitman, W. C., III, Ryan, W. B. F., and Bonnin, G., *Bull. geol. Soc. Am.*, 84, 3137-3180 (1973).

⁵ Radulescu, D. P., and Sandulescu, M., *Nature*, 257, 69 (1975).

⁶ Gavai, I., Ciupasea, D., and Airinei, St., *Acta geol. hung.*, 13, 183-190 (1969).

⁷ Stegena, L., *Geothermics*, 1, 140-141 (1972).

⁸ Stegena, L., Géczy, B., and Horvath, F., *Tectonophysics* (in the press).

⁹ Roman, C., *Nature*, 228, 1176-1178 (1970).

LSD and dopamine receptors

PIERI *et al.*¹ reported a circling behaviour response to LSD in rats with unilateral chemical lesions of the ascending medial forebrain bundle. They used two rotational models; one was produced by 6-hydroxydopamine injection and the other by 5,6-dihydroxytryptamine injection. The factor common to both models was a deficit of dopamine in the ipsilateral forebrain. The authors demonstrated strong maximal contralateral turning responses to LSD given in a dose range of 0.1-1.5 mg kg⁻¹. Only the time course of turning seemed to be dose dependent.

We have repeated these experiments in similar animal models and have found the effects less convincing. Indeed, LSD

¹ Boccaletti, M., Guazzone, G., *Nature*, 252, 18 (1974).

produced apomorphine-like (contraversive) circling behaviour in only 40–50% of animals tested, and then at the very high drug levels of 1 and 1.5 mg kg⁻¹. In the turning mouse model of von Voigtlander and Moore², where 1 mg kg⁻¹ apomorphine induced a mean rate of turning of 5 min⁻¹, no satisfactory data for LSD could be obtained. In the dose range 0.025–0.2 mg kg⁻¹, LSD induced neither turning behaviour or postural asymmetries nor significantly modified apomorphine or amphetamine-induced circling, as may be predicted were it a potent dopamine agonist. Where rotation was induced following administration of 1.5 mg kg⁻¹ LSD the duration was of the order of 35–45 min; not 2 h as Pieri *et al.* suggest. We agree with them that rotation was prevented by previous treatment with haloperidol (0.5 mg kg⁻¹), and not after α -methyl-*p*-tyrosine (250 mg kg⁻¹).

In another experimental animal model, namely, audiogenic seizures in inbred strains of mice, dopamine agonists have been shown to diminish the severity of the seizure response^{3,4}. In our experiments, apomorphine was at least 10 times more potent than LSD in blocking the clonic phase of the seizure in 50% of animals.

The pharmacology of LSD is very confused, but has previously been associated with central 5-HT neurones^{5–7}. Pieri *et al.*, however, have claimed a direct and potent action of LSD on the dopaminergic receptor *in vivo*. Our data do not confirm LSD as being a potent dopamine agonist, although it seems to be capable of stimulating the dopamine receptor in very high doses.

CHRIS PYCOCK
GILL ANLEZARK

Department of Neurology,
Institute of Psychiatry,
De Crespigny Park,
London SE5 8AF, UK

¹ Pieri, L., Pieri, M., and Haefely, W., *Nature*, **252**, 586–588 (1974).

² Von Voigtlander, P. F., and Moore, K. E., *Neuropharmacology*, **12**, 451–462 (1973).

³ Lehmann, A., in *Physiological Effects of Noise* (edit. by Welch, B. L., and Welch, A. S.), 227–257 (Plenum, New York, 1970).

⁴ Anlezark, G. M., and Meldrum, B. S., *Br. J. Pharmacol.*, **53**, 419–421 (1975).

⁵ Andén, N. E., Corrodi, H., Fuxe, K., and Hökfelt, T., *Br. J. Pharmacol.*, **34**, 1–7 (1968).

⁶ Chase, T. N., Breese, G. R., and Kopin, I. J., *Science*, **157**, 1461–1463 (1967).

⁷ Boakes, R. J., Bradley, P. B., Briggs, I., and Dray, A., *Br. J. Pharmacol.*, **40**, 202–218 (1970).

PIERI ET AL. REPLY—The results reported¹ correspond to a well defined model, namely rats unilaterally lesioned in the medial forebrain bundle with 5,6-HT or 6-OHDA. These two types of lesion have been extensively investigated with biochemical^{2,3} and histofluorescence methods (H. P. Lorez, unpublished), and result in a marked and long-lasting depletion of dopamine (DA), possibly leading to the subsequent development of denervation supersensitivity, (1 mg kg⁻¹

apomorphine being able to induce more than 15 turns min⁻¹ for 30 min). Lesioned animals were challenged with apomorphine and only those responding with a clear circling (about 50–60%) were subsequently used for the study of the effect of other agents, including LSD (80% of responses). The data obtained with several hundreds of rats have been described in part previously⁴ and a more extensive paper is in the press⁵. The fact that Pycock and Anlezark⁶ seem to have used a different animal species and a different lesion, clearly yielding less denervation supersensitivity (1 mg kg⁻¹ apomorphine inducing a mean rate of turning of 5 min⁻¹), precludes any reasonable comparison.

Concerning the relative potencies of apomorphine and LSD as striatal DA receptor agonists, it seems from our data that LSD is slightly more potent than apomorphine. By no means, however, do we claim that this applies to completely different models such as that reported by Pycock and Anlezark⁶ (namely, audiogenic seizures in mice).

It should also be stressed that low doses of LSD elicit a significant slowing of DA turnover in rat striatum (0.2 mg kg⁻¹ intraperitoneally) and retina (0.5 mg kg⁻¹ intraperitoneally). In addition, the striatal adenylate cyclase is activated by concentrations of LSD as low as 10⁻⁶ M (ref. 7). Thus, there is also biochemical evidence for DA receptor stimulating properties of LSD.

We did not intend to suggest that the DA receptor stimulant effect of LSD observed in our experimental conditions should be the final answer to the confused pharmacology of the compound. The DA-like component of LSD action in the central nervous system is, in our opinion, additive and not alternative to the stimulant action of the compound on 5-HT receptors. In circling behaviour, however, DA receptor stimulation seems to be important, at least in our model⁵.

L. PIERI
M. PIERI
W. HAEFELY

F. Hoffmann-La Roche & Co. Ltd,
4002 Basel, Switzerland

¹ Pieri, L., Pieri, M., and Haefely, W., *Nature*, **252**, 586 (1974).

² Saner, A., Pieri, L., Da Prada, M., and Pletscher, A., *Experientia*, **30**, 697 (1974).

³ Saner, A., Pieri, L., Moran, M., Da Prada, M., and Pletscher, A., *Brain Res.*, **76**, 109 (1974).

⁴ Pieri, L., and Pieri, M., *Experientia*, **30**, 696 (1974); *J. Pharmacol. (Paris)*, Suppl. 2, 5, 77 (1974).

⁵ Pieri, M., Pieri, L., Saner, A., Da Prada, M., and Haefely, W., *Archs int. Pharmacodyn.* (in the press).

⁶ Pycock, C., and Anlezark, G., *Nature*, **257**, 69–70 (1975).

⁷ Da Prada, M., Saner, A., Burkard, W. P., Bartholini, G., and Pletscher, A., *Brain Res.* (in the press).

Mimetic talking by parrots

THE paper by Gregory and Hopkins¹ has prompted me to speculate on the question; What is the biological sig-

nificance (or survival value) of 'talking' by parrots? Of course, parrots do not actually talk, they only imitate speech. In fact, they imitate, not only speech, but many sorts of discernible sound patterns that are likely to be produced repeatedly in their environment. Indeed, the articulated sounds, I suggest, signify a kind of 'functional' mimicry, whereby an animal is camouflaged by becoming one more source of its natural environmental noise. Yet, the animal does not presumably utter the mimetic sound patterns at random times and spaces, but rather it may utter them as responses to specific outer signals. This behaviour implies the existence of integrated neuronal circuits; and as a dominant role of the visual system is known in birds², the pupil constriction observed, suggests that certain (learned) visual patterns may act as signals (stimuli) that trigger talking behaviour. Thus, pupil constriction may serve to obtain clearer, optimal images of such visual patterns. Guzmán-Flores (personal communication) has found that parrots fail to learn recurrent, tape-recorded utterances, in which the correlative significant visual stimuli are missing.

F. ALONSO DE FLORIDA

Facultad de Medicina,
Departamento de Fisiología,
Apartado Postal 70-199,
Ciudad Universitaria,
México DF, Mexico

¹ Gregory, R., and Hopkins, P., *Nature*, **252**, 637–638 (1974).

² Polyak, S., *The Vertebrate Visual System* (University of Chicago Press, 1957).

Hereditary persistence of foetal haemoglobin

ALTHOUGH the interpretation of Martinez and Colombo¹ may be correct, their conclusions are based on uncertain evidence and an alternative explanation fits within the classical scheme is equally plausible. Their argument rests entirely on the statement that "foetal haemoglobin (HbF) levels show intrafamilial segregation in β^{thal} ". From this statement, they conclude that III₂ cannot simply be a β^{thal} heterozygote like I₂. Our experience shows that this is not invariably so: parents, offspring, and siblings who are β^{thal} heterozygotes may differ in level of HbF. Consequently, we believe that III₂ is only a β^{thal} heterozygote and that his slightly elevated HbF is the result of his particular expression of the condition. The inheritance pattern then follows normally. I₁, II₁, and III₁ all have a chromosome with S and hereditary persistence of foetal Hb (HPFH); II₁ also has β^{thal} ; III₁ and III₂ have a normal chromosome from II₂.

The presence of HbS and HPFH in the *cis* form has not been reported, but is entirely feasible from our knowledge of the genetics and arrangement of the complex of β , γ , and δ genes². A type of HPFH which has only about 5% HbF has been described³. We have recently examined a family in which, in contrast to other HPFH classes, β^A chains are produced in *cis* to HPFH (ref. 4) and have speculated that a type of HPFH with production of β^S chains in *cis* is possible. Martinez and Colombo may have detected this combination. They report, however, a heterogeneous intracellular distribution of HbF in I₁ and III₁, whereas a homogeneous distribution is one of the criteria of HPFH. Consequently, it is more probable that I₁ and III₁ merely have sickle cell trait with somewhat elevated HbF.

In summary, we believe that Martinez and Colombo have misjudged the genetic aspects of several members of this family and have made unwarranted interpretations. In all probability, I₁ and III₁ have sickle cell trait, I₂ and III₂ have β^{thal} trait, and II₁ has S- β^0 -thal.

W. A. SCHROEDER

Division of Chemistry and Chemical Engineering,
California Institute of Technology,
Pasadena, California 91125

T. H. J. HUISMAN

Medical College of Georgia,
Augusta, Georgia 30902

¹ Martinez, G., and Colombo, B., *Nature*, **252**, 735 (1974).

² Huisman, T. H. J., et al., *Ann. N.Y. Acad. Sci.*, **232**, 107 (1974).

³ Sukumaran, P. K., et al., *Br. J. Haemat.*, **23**, 403 (1972).

⁴ Huisman, T. H. J., Miller, A., and Schroeder, W. A., *Am. J. Human Genet.* (in the press).

DRS MARTINEZ AND COLOMBO REPLY—The argument put forward by Schroeder and Huisman¹ in their criticism to our paper² rests entirely on the sentence: "it is more probable that I₁ and III₁ merely have sickle cell trait with somewhat elevated HbF (foetal haemoglobin)", as confirmed by the last sentence of their letter. With this statement they want to imply that the elevation of HbF is determined by some non-genetical factor(s). This means that to interpret the elevated percentages of HbF in I₁, III₁ and III₂ the following assumptions must be made. A subject and his grandmother, both showing HbS trait only, have exceptionally elevated percentages of HbF which are exactly the same (and by definition are not genetically determined); and a simple β^{thal} carrier is doubly exceptional because this subject not only shows HbF levels exceptionally high for this condition³, but also these levels do not show the intrafamilial segregation

reported by many authors (see, for example, refs 3 and 4). We consider that it must be very exceptional as only the opposite situation has been reported in the literature.

Being somewhat reluctant to postulate so many unwarranted hypotheses, we preferred to propose just one, that is, that in our family there was a segregation of a type of hereditary persistence of foetal haemoglobin (HPFH).

If we are in the presence of a type of HPFH the hypothesis that this HPFH is in *cis* to β^S (as mentioned and rejected also by Schroeder and Huisman) cannot be accepted for the following reasons: the ratio β^S/β^A was normal in I₁ and III₁; the gamete transmitted from II₁ to III₂ should be a recombinant between HPFH and β^S .

Thus the fact that the gene for this HPFH could not be in *cis* to β^S nor to β^A (see pedigree) enabled us to claim that we were in the presence of a "new type of HPFH".

It is evident then that the objection raised by Schroeder and Huisman concerning the intracellular distribution of HbF becomes completely irrelevant.

In summary, we believe that Schroeder and Huisman have misjudged the genotypes of all the members of this family showing elevated percentages of HbF. In fact trying to reject the most logical interpretation of our findings, they were forced to discard *tout-court* the possibility that this persistence of HbF was hereditary, thus creating for each member of the family the unwarranted, although unexpressed, number of necessary hypotheses.

Instituto de Hematologia e Immunologia,
Habana 8, Cuba

¹ Schroeder, W. A., and Huisman, T. H. J., *Nature*, **257**, 70–71 (1975).

² Martinez, G., and Colombo, B., *Nature*, **252**, 735 (1974).

³ Weatherhall, D. J., and Clegg, J. B., *The thalassaemia Syndromes* (Blackwell, Oxford, 1972).

⁴ Friedman, S., Hamilton, R. W., and Schwartz, E., *J. clin. Invest.*, **52**, 1453 (1973).

Ultraviolet light and human cataract

WEITER and Finch¹ could find no difference in paramagnetic species between normal and cataractous human lenses but demonstrated that prolonged ultraviolet irradiation of normal human lens produced free radical species, which they presumed were derived from tryptophan. They suggest that the production of free radicals by ultraviolet light might be a mechanism for photoinduced lens damage.

The theory that the ultraviolet radiation of sunlight (or from other sources) can cause brown nuclear cataract has been the subject of prolonged discussion, but there are two major arguments against such a

theory²: (1) The proteins of the brown cataractous nucleus do not show a loss of tryptophan compared with the normal human lens², whereas a substantial loss of tryptophan is found in the products of *in vitro* photo-oxidation of lens proteins³; and, (2) in brown nuclear cataract, only the lens nucleus is pigmented and it is difficult to see how ultraviolet light could act on proteins of the lens nucleus alone, rather than those of the cortex, which are very similar; especially as absorption of ultraviolet by the cornea and outer layers of the lens would ensure that little harmful radiation could reach the centre of the lens.

KEITH J. DILLEY

Nuffield Laboratory of Ophthalmology,
University of Oxford,
Walton Street, Oxford OX2 6AW, UK

¹ Weiter, J. J., and Finch, E. D., *Nature*, **254**, 536–537 (1975).

² Dilley, K. J., and Pirie, A., *Expl. Eye Res.*, **19**, 59–72 (1974).

³ Buckingham, R. H., and Pirie, A., *Expl. Eye Res.*, **14**, 297–299 (1972).

WEITER AND FINCH REPLY—The invariance of tryptophan content in normal and cataractous lenses as opposed to the loss of tryptophan in the *in vitro* photo-oxidation of lens proteins may result from the likelihood that the tryptophan in the lens proteins *in vivo* may absorb the radiation and transmit the energy to some other species (for example, lipids) to produce the radical, which may in turn cause damage to the lens. If this is so, the tryptophan would remain essentially unaffected. Steen¹ has shown that tryptophan in an ethylene glycol–water glass at 77 K absorbs ultraviolet light to produce the radical in the solvent, and tryptophan itself may not form the radical. The growth of radical, however, in both Steen's and our own (unpublished) experiments with lens material is not linear with respect to exposure time, thereby indicating the possibility of some degradation of tryptophan and its consequent unavailability for excitation. It is likely that this could be the result of a mechanistic detail rather than tryptophan degradation. Production of a radical through tryptophan excitation may involve several steps and this may diminish the efficiency of radical production.

The second point we would make is that Steen¹ has pointed out that the formation of free radicals by ultraviolet light on tryptophan in ethylene glycol–water glass is accompanied by significant coloration of the sample and this was attributed to trapped electrons. The trapping was most efficient in the presence of a substantial concentration of H⁺ ions, which are known to scavenge electrons very well. If we assume that the cataract coloration results from trapped electrons, a differential cationic concentration between the cortex and the nucleus could explain this nuclear pigmentation through a colour

centre formation. Alternatively, the coloration may arise from the formation of stable lipid peroxides, although in this case, we do not know why the nucleus is favoured discriminatingly for the production of lipid peroxide radicals.

J. J. WEITER
E. D. FINCH

Naval Medical Research Institute,
Bethesda, Maryland, 20014

¹ Steen, H. B., *Photochem. Photobiol.*, 9, 479 (1969).

Reversibility and biological machines

GRAY¹, in discussing reversibility and biological machines, applied a formula derived by Brillouin², describing the energy required to determine the positional limits of a microscopic system, to the muscle cross bridge. A result of his analysis is that the cross bridge is too small to be controlled directly by any internal system which matches the tension to the external load, and that control must extend over greater distances; I doubt if anyone would contest this. Gray then, however, extends the concept of 'profitable controllability' from this restricted example to all machines of molecular dimensions which operate cyclically and argues that their mode of operation involves an irreversible, unidirectional step; if this is not so, then the efficiency of energy conversion must be very low indeed. This idea is identical to that of McClare³, who has argued that muscle contraction is an irreversible quantum-mechanical process whereby the free energy from ATP is converted to mechanical work. In fact, perfectly workable, self consistent models of muscular contraction can be made without any recourse to quantum mechanics, by general methods applicable to any energy conversion process⁴, but Gray claims to advance a definite proof that this is impossible and that biological molecular machinery cannot be considered as an energy converter in the classical thermodynamic sense.

This does not follow, however, from Gray's idea of 'profitable controllability' which only has meaning when the rate of working of a molecular machine is geared to the prevailing force-field (thermodynamic gradients or whatever) by some cybernetic process, to achieve a thermodynamically reversible operation. If this criterion does not apply the 'controllability' of the system is meaningless, or rather irrelevant. There is no evidence that molecular machines must be controlled in this way, and so Gray has only produced a proof for one highly restricted set of conditions. It is perhaps worth considering the case of such molecular machines

as ion pumps in the cell membrane, which can be driven backwards and which seem to function with high efficiency⁵. It is not possible to argue that there is no direct energy conversion occurring (as in a system involving mechanical work) because a substantial part of the work performed is that of moving a charge through an electric field gradient; that is, free energy from ATP is being used directly, by way of a conformational change, to achieve translation in a force-field. This must be taken to be a direct experimental disproof of Gray's thesis.

In summary, although the rate of energy conversion by a biological machine of molecular dimensions is subject to microscopic variation within the statistical limits imposed by the Second Law, there is no proof that size alone has any direct connection with the overall efficiency of the energy conversion process, or its potential reversibility.

A. E. HILL

Botany School,
University of Cambridge,
Cambridge CB2 3EA, UK

¹ Gray, B. F., *Nature*, 253, 436 (1975).

² Brillouin, L., *Science and Information Theory* (Academic, New York, 1962).

³ McClare, C. W. F., *Nature*, 240, 88 (1972).

⁴ Hill, T. L., *Prog. Biophys. molec. Biol.*, 28 (1975).

⁵ Garrahan, P. J., and Glynn, I. M., *J. Physiol., Lond.*, 192, 217 (1967).

GRAY REPLIES—In the first few lines of his comment Hill¹ incorrectly paraphrases the contents of my letter, then identifies his version with a theory of McClare² (to which I did not even refer) which has been published for some time. He attributes to me claims which I do not make, for example, to advance a definite proof that "perfectly workable self-consistent models of muscular contraction cannot be made without recourse to quantum mechanics". Perfect workability, however, depends on how much or how little detail one is satisfied with in a theory. Hydrogen and oxygen will react explosively to form water

and the statement $\Delta G < 0$ and its implications may be a 'perfectly workable self-consistent model' to some people who are interested only in distinguishing potential explosives from inert material. To the man interested in why the reaction is explosive, at one temperature and almost immeasurably slow at a temperature one degree lower, however, this 'perfectly workable' model is completely useless! He needs the macroscopic theory of chain reactions, and a third man with an even greater sense of curiosity needs quantum mechanics to understand the magnitudes of the reaction cross sections involved in this theory.

The first part of Hill's second paragraph reveals a misunderstanding of my main thesis, which is that very small (molecular) machines cannot be individually controlled if they are to work efficiently, not the reverse. He argues that there is no evidence that biological molecular machines are controlled "by gearing to some prevailing force field to achieve a thermodynamically reversible operation". He is here reiterating the view expressed by McClare², and my letter shows this is necessarily true, from a consideration of quantum theory and the observed sizes and efficiencies of molecular machines. We seem to agree that the independent working units, such as a crossbridge or a pump site, cycle autonomously in normal conditions in their forward direction, that is, using ATP. Clearly they cannot be made to go in reverse without the intervention of control, by definition, thus giving a concomitant drop in efficiency for units of this size. Experimental results on the reversal of ion pumps³ do not conflict with this conclusion, since the reversal of the ion pump obtained in human red cells was achieved in highly abnormal conditions, not the normal environment of these cells and the energy expended in producing this abnormal environment must not be ignored in any discussion of efficiency and reversibility. Obviously any cyclic machine can be run backwards if one goes to sufficient lengths to push it, but there will be a drastic loss in efficiency except in the classical limit, where one can operate reversibly without energy cost. My brief statement about the reversal of muscle should be understood in this sense.

Hill's last statement does not seem to make sense, as the Second Law imposes no limits, statistical or otherwise, on the rate of energy conversion of any machines from molecular biological to macroscopic steam engines. It gives a criterion for distinguishing processes in which work can potentially be extracted from others in which it cannot.

B. F. GRAY

University of Leeds

¹ Hill, A. E., *Nature*, 257, 72 (1975).

² McClare, C. W. F., *J. theor. Biol.*, 30, 20 (1971).

³ Glynn, I. M., Lew, V. L., and Luithi, U., *J. Physiol., Lond.*, 207, 371 (1970).

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

reviews

RIBOSOMES are subcellular organelles that are found mainly in the cytoplasm. They are important because they are the site of protein biosynthesis, where the linear nucleotide sequence of messenger RNA is translated into the linear amino acid sequence of a particular protein. They may be regarded as multifunctional multisubunit enzymes and they are unusual because they contain RNA as a major component.

Ribosomes were first isolated and studied about 25 years ago. James Watson and Alfred Tissières began the study of the bacterial ribosome in the late 1950s and the complexity of the particle (at least 3 RNA species and at least 55 different protein species) began to emerge. For a time, during the period when the amino acid code was being solved, it was convenient to regard the ribosome as a black box. Around 1968 several developments took place that allowed a study of the black box itself. These developments included: the isolation of individual ribosomal proteins; the reassembly of functional subribosomal particles from their RNA and protein components; the preparation of antibodies to individual proteins and the invention of other techniques for analysing complicated protein mixtures; the emergence of nucleotide sequencing techniques; and the choice throughout the world of *Escherichia coli* ribosomes as the preferred system for study—this concentration on a single species has proved specially important—not least in promoting collaborative research.

This book arises from a meeting on ribosomes held in Cold Spring Harbor in September 1973 and fixes the view of the bacterial ribosome (particularly the *E. coli* ribosome) as it appeared at the meeting. The book has many admirable

Ribosomes

R. A. Cox

Ribosomes. By M. Nomura, A. Tissières and P. Lengyel. Pp. x+930. (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1974.) \$32.00.

features. The first part comprises a series of general reviews. The editors have carefully chosen the contributors from those invited to the meeting, and a few who were themselves the pioneers responsible for some of the most recent advances were asked to provide more general surveys of topics such as RNA-protein interactions in the ribosome, the protein topography of ribosomal subunits, and the reconstitution of ribosomes, as well as reviews of physical properties of ribosomes, and of the isolation and properties of RNA and proteins. The process of translation, ribosome genetics and ribosome biosynthesis are also reviewed. The reviewers generally produced more than a factual survey and have emphasised where possible the relationship between structure and function. By no means all of the progress achieved in ribosome research has a chemical basis but a major impression remains that a chemical approach has proved to be fruitful for probing ribosome structure and eroding the 'black box' concept. For example, it can now be said "that the fidelity with which messenger RNA can be translated depends critically on the amino acid residues at positions 42 and

87 of protein S12". Bifunctional cross-linking agents have proved useful in identifying those proteins that are neighbours in the ribosome, and affinity labelling methods have been used increasingly to identify functionally active groups.

The second part of the book is made up of a series of carefully selected "Specific Reviews" of topics such as electron microscopy, affinity labelling techniques, immunochemical analysis, fluorescence studies, ribosome genetics, "magic spot", RNA synthesis *in vivo* and *in vitro* and particular aspects of ribosome function. There are three specialist reports on antibiotics and the ribosome but no general review of this topic although widespread use is made of these substances as probes of ribosome structure.

The glory belongs to the *E. coli* ribosome but about a fifth of the book is devoted to ribosomes of eukaryotes. There is one chapter and one specific report devoted to eukaryotic ribosome structure. This emphasis is, however, a fair reflection of the field as a whole.

The book comprises 930 pages and a comprehensive index. It provides a very, clear picture of the *E. coli* ribosome as it was seen in late 1973 with the proteins preeminent and the RNA moiety in the shadows; and it also reveals and documents the remarkable progress made over a five-year period. It is a monumental effort that does justice to the subject—indeed, it is the most ambitious publication in the field so far—and the standard of precision and clarity it achieves in giving shape to so many diverse facts is very high. This book has much to offer those who are interested in nucleoproteins and it is compelling reading for those who are actively interested in ribosomes.

Aromaticity

A. W. Johnson

Facts and Theories of Aromaticity. By David Lewis and David Peters. Pp. viii+109. (Macmillan: London and Basingstoke, April 1975.) £10.00.

following up any particular aspect. After a brief historical introduction, chapters are given on experimental evidence,

theoretical ideas and simple examples of aromaticity and a variety of non-benzenoid structures: for example, quinoids, pyrones and porphyrins are also considered. Finally, ideas on homoaromaticity and antiaromaticity are summarised and some general conclusions are advanced. It is an interesting text on a topic of interest to all organic chemists and it is well written and produced in an attractive format, as indeed would be expected for about 10 pence a page.

THE concept of aromaticity in organic molecules, which has done so much to inspire experimental work in both mechanistic and preparative areas, is still the subject of much debate and controversy. The present volume sets out to present the varied aspects of aromaticity in a critical and concise manner and the result is a very readable account of the present status of the subject. What is lost in detail in the text is compensated for by an extensive list of references so that the reader will have no difficulty in

Books for Autumn 1975

Fish Communities in Tropical Freshwaters

R H Lowe-McConnell

By reference to ecological studies in freshwater fishes, this book illustrates how tropical fish communities differ from those in temperate waters. The book makes available to the general ecologist the results of recent research into the ecology of the fish.

Although the book is intended primarily for college and university students it will be of interest to the general reader concerned with ecological concepts, zoogeography or fish biology.

£10.00 net

Forthcoming:

Guidebook to Stereochemistry

F D Gunstone

This guidebook provides a foundation for the understanding of stereochemistry - a subject which is of fundamental importance in all sciences studied at the molecular level. As most molecules exist in three dimensional form, molecular shape must be understood in order to rationalise and predict chemical and biological behaviour. There are chapters dealing with *cis-trans* isomerism, enantiomerism, conformation, and dynamic stereochemistry.

£2.95 net

Forthcoming:

Heterocyclic Chemistry

D W Young

An introduction to the chemistry of heterocyclic compounds for students of chemistry, pharmacy and biochemistry, who possess a basic knowledge of biochemistry. The reactions of heterocyclic compounds are compared with those of their simple organic-functional group analogues by noting how ring and heteroatoms interact to produce the unique properties exhibited by heterocyclic compounds.

The chemical effects of aliphatic heterocyclic compounds are discussed, and consideration is given to aromaticity and tautomerism, leading to a discussion of hetero-aromatic compounds in general.

Probably £3.50 net



Longman

Quantum optics

Introduction to Quantum Optics. (Documents on Modern Physics.) By H. M. Nussenzveig. Pp. xiv + 246. (Gordon and Breach: London, New York and Paris, December 1974.) DM 59; \$24.40.

Optical Resonance and Two-Level Atoms. (Interscience Monographs and Texts in Physics and Astronomy, vol. 28.) By L. Allen and J. H. Eberly. (Wiley Interscience: New York and London, March 1975.) £10.80.

ALTHOUGH the range of topics discussed in these books is not identical there is, nevertheless, some relationship between them. One obvious link is that Professors Nussenzveig and Eberly are both in the same department of the University of Rochester. Furthermore, the books are of comparable length and depth, and are clearly intended for the same type of audience. And both include chapters on superradiance, a topic that has so far received little text book coverage.

In *Introduction to Quantum Optics*, the foundations of the subject are treated in a fairly standard way. The bulk of the book is based on a series of lectures presented by the author at the Latin American School of Physics in La Plata in 1970, and is devoted to the classical and quantum theories of optical coherence and the semiclassical and quantum theories of the laser. A chapter has since been added on coherent interactions, in which superradiance, photon echoes and self-induced transparency are described from a semiclassical viewpoint. The book closes with a rather disjointed 30 page appendix on recent developments (much of which is devoted to the quantum theory of superradiance), which certainly serves to emphasise the unfinished nature of quantum optics research. The style throughout is very readable and there are adequate references at the end of each chapter.

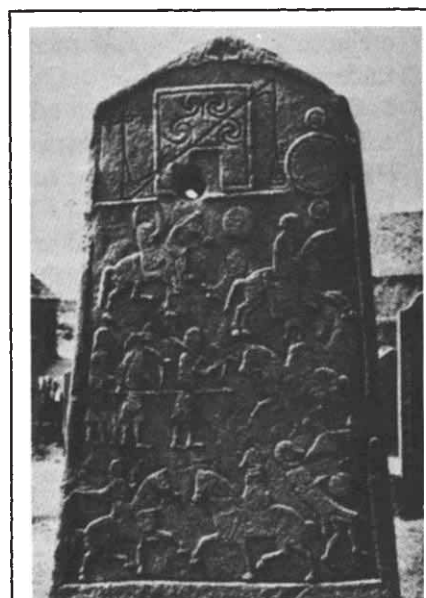
The book is by no means unique in its coverage, apart from perhaps the welcome inclusion of superradiance. In his introduction, Professor Nussenzveig states that the book is "aimed at theoretical physicists and graduate students . . . as an introduction to a new subject with which no previous familiarity is assumed". One happy consequence is that the level of presentation is not so high that the experimentalist will immediately be daunted. In this, perhaps, lies the book's particular merit.

Optical Resonance and Two-Level Atoms is more original in conception, bringing together material that has until now been widely scattered in other books or reported only in research journals. Indeed, it forms an excellent sequel to *Introduction to Quantum Optics*, being devoted entirely to the resonance phenomena treated in the last chapters

of Professor Nussenzveig's book. Both authors have published extensively in this field although the stated intention of the book is not to emphasise recent research but rather to reveal the general principles involved. The first chapter deals with the classical theory of absorption from an unconventional point of view which allows the authors to introduce the idea of pulse "area". An examination of the validity of the two-level assumption and a useful discussion of the various other approximations commonly used leads on to an extended treatment of Bloch vector dynamics and self-induced transparency, which forms the central section of the book. Experimental results are compared with the theoretical predictions, the possible confusion of self-induced transparency with incoherent saturation effects being carefully explained. A chapter on single-atom spontaneous emission using quantised field theory lays the groundwork for the discussion of cooperative phenomena, superradiant decay and photon echoes which forms the second part of the book. The style of writing is clear and informal and the emphasis throughout is always on the physics of the processes taking place. There are numerous helpful illustrations and adequate lists of references.

Both *Introduction to Quantum Optics* and *Optical Resonance and Two-Level Atoms* represent welcome additions to the literature on quantum optics. The latter will prove particularly valuable because of its unusual coverage, although both can be warmly recommended.

G. H. C. New



Pictish horse and foot soldiers form a battle scene on the back of a Pictish sandstone cross slab, Aberlemno churchyard, Aberlemno, Angus, Scotland. Taken from *Scotland: An Archeological Guide* by Euan W. Mackie. Pp. x + 298. (Faber and Faber: London, June 1975.) Cloth £5.50; paper £2.95.

Seismic Waves. By E. F. Savarenskii. Translated from Russian. Pp. vi+281. (Israel Program for Scientific Translations: Jerusalem; Wiley: Chichester; May 1975.) £13.95.

THIS book, excellently translated, caters mainly for the physicist who wants to study elastodynamics and that part of data-handling needed with seismic data. There are three chapters: the first, on oscillations, deals mainly with Fourier analysis and operations in the frequency domain; the second, on waves, derives the basic equations of elastic wave motion and goes on to obtain radiation fields for point sources in an unbounded medium; the third describes the propagation of plane seismic waves in a half-space or a layer.

The book is written at a fairly elementary level, and mathematical demonstrations are sketchy. It omits, for instance, mention of the splitting of degenerate eigenfrequencies of the Earth, or of matrix methods for layered media. The reader will not find such recent topics as earthquake prediction, lunar seismology or deep seismic sounding. Within its scope, Savarenskii's book is clear and practical; it has obviously grown out of the experience of an expert seismologist. **E. R. Lapwood**

Bee Research Association, 1949-1974: A History of the First 25 Years. Pp. 199. (Bee Research Association: Gerrards Cross, 1974.) np.

LAST year was the 25th anniversary of the founding of the Bee Research Association and to help commemorate the first quarter of a century a history of the association, to which many of its prominent members have contributed, has now been published.

The history traces the foundation of the Bee Research Association as an offshoot of the research committee of the British Beekeepers' Association, its growth in size and importance to an international organisation and its acquisition of a permanent headquarters at Hill House in Chalfont St Peter. During this process it took over publication of the well established journal *Bee World*, introduced *Apicultural Abstracts*, first as an integral part of *Bee World* in 1950 and then as a separate quarterly journal in 1962, and launched *Journal of Apicultural Research* in 1961 in response to a strongly felt need for a journal devoted to experimental work relating to pollination and beekeeping.

Bee research workers and advisory officers readily acknowledge the value to their work of these three journals and the many other facilities the association provides, including its library of re-

prints, books and translations, all of which are discussed at length in this book. With the recent increase in beekeeping and in the use of bees for pollination, especially in developing countries, the services of the association are in greater demand than ever before. It is vital that they should continue to grow.

John B. Free

Books brief

Atomic Physics. (The Manchester Physics Series.) By J. C. Willmott. Pp. xiv+357. (Wiley: London and New York, April 1975.) £8.50 cloth; £4.25 paper.

THIS textbook is intended for the second year of the honours physics course at an English University, specifically, Manchester, whose reputation in atomic and molecular physics is high. The book contains the necessary quantum mechanics, and inevitably, material that might be taught in the first year, and material that would in the opinion of some belong in the final year. But it stops short of Lamb shift and hyperfine structure. The author is primarily a nuclear physicist, and it is to some extent a reflection on atomic physicists that the subject is not particularly well served by undergraduate texts; for this reason, the book will fulfil a useful role. It is a competent book, although not everyone will find it inspiring. There are sets of problems in each chapter, and it is well produced and indexed.

J. B. Hasted

Registry of Mass Spectral Data; volumes 1-4. Edited by E. Stenhagen, S. Abrahamsson and F. W. McLafferty. Pp. xvii+3,358 (Wiley, New York, 1974.) £250.00.

WHEN it was first developed and became commercially available in the early 1960s, mass spectrometry was hailed as the panacea that would take the pain out of structure elucidation. No longer would organic chemists have to slave for months, if not years, degrading and analysing new compounds; instead a mass spectrum would tell all, and even a milligram of material would be sufficient. But this early euphoria was not wholly justified, and since then in the years of appraisal that followed, the limitations and the complications of mass spectrometry have become apparent. Although the expert practitioner, backed up by expensive and elaborate computing facilities, is able to

combine mass spectral fragments and deduce the structure of his unknown, the average organic chemist can deploy neither expertise nor facilities on this scale. Instead, he merely compares the spectrum of his unknown with those of known compounds and attempts to spot significant similarities.

This is where the *Registry of Mass Spectral Data* fits in. It is an immense and invaluable collection of reproductions of 18,806 authentic mass spectra. The spectra themselves are easy to locate, being arranged in order of increasing molecular weight (to the nearest whole mass unit); there is also an excellent index. Although expensive, the cost is, in fact, less than two pence a spectrum, and when the price of even the most modest mass spectrometer is considered, the additional expense in purchasing this unique compendium of spectra as an accessory is easily justified. I strongly recommend the *Registry of Mass Spectral Data* as a must for every laboratory in which mass spectrometry goes on; it cannot afford to be without one.

E. J. Thomas

Biological Transport. Second Edition. By Halvor N. Christensen. Pp. xvi+514. (Benjamin: Reading, Massachusetts and London, April 1975.) \$19.50.

NOWADAYS, the term 'Biological Transport' concerns the nature of, and mechanisms underlying, the transport of substances between different phases separated by membranes (for example, plasma and mitochondrial) or by epithelial structures (for example, intestine, placenta, renal tubule).

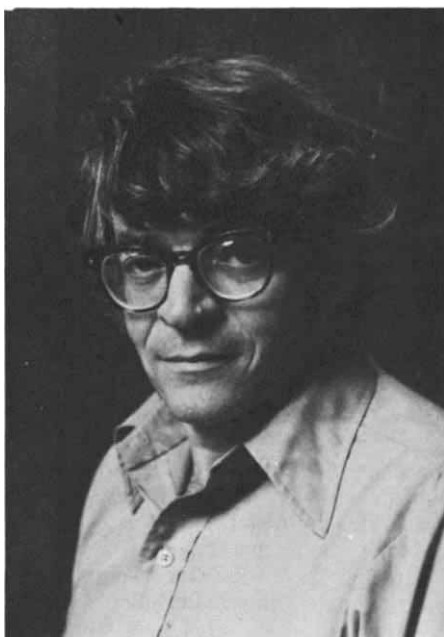
This second edition of H. N. Christensen's excellent book is really a complete new work and the author is to be congratulated on attempting to embrace within one volume all aspects of epithelial transport and membrane structure and function, including kinetic, thermodynamic and genetic aspects of real and artificial systems. He has largely succeeded in surmounting the disciplinary prejudices that seem to have caused an exceptional degree of fragmentation in the subject, and the book will accordingly be useful to workers studying membranes and epithelia.

The account of the ATPases seems somewhat old fashioned; vitamin B₁₂ is not absorbed in the duodenum; the electron micrograph of the erythrocyte is an antique; and after page 367 the contents page and the text become out of phase. But there is much in this book for those interested in any aspect of biological transport, and that must include biochemists, morphologists, physiologists and molecular biologists.

Dennis S. Parsons

obituary

Gordon Mayer Tomkins of the University of California, San Francisco, one of America's most distinguished biochemists and a pioneer research scientist in the field of hormone activity, died in New York City, July 22, 1975. He was 49 years old. He had been ill since undergoing brain surgery in late May.



To his colleagues in scientific institutions all over the world Dr Tomkins was an extraordinary individual. He was a physician, a highly creative scientist, a teacher of unparalleled enthusiasm and an accomplished musician who played both classics and jazz with distinction.

Dr Tomkins was born in Chicago and received an A.B. degree in Philosophy from UCLA, an M.D. degree from Harvard Medical School and a Ph.D. degree from the University of California, Berkeley. He spent 16 years at the NIH, Bethesda, Maryland, and left there in 1969 as Chief of the Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, to join the University of California, San Francisco, as Professor of Biochemistry.

He served on numerous editorial boards for scientific journals and

periodicals and was the recipient of many honours, including the Mider Lectureship (NIH, 1969); the Jesup Lectureship (Columbia, 1971); the Harvey Society Lectureship (Rockefeller, 1972); the Prather Lectureship (Harvard, 1972); and the Baker Lecture (Cornell, 1975).

His scientific contributions encompass broad aspects of biological chemistry and molecular biology. His first publications described the regulation of cholesterol biosynthesis by diet. From this early involvement with sterols developed his intense interest in steroid hormones and their molecular mechanisms of action. He described the now important enzymatic reductions and hydroxylations of steroids and soon after introduced the concept of and provided evidence for a low molecular weight compound, such as a steroid hormone, specifically changing the conformation of a protein molecule. This concept, subsequently termed *allostery* by others, is an important one not only in endocrinology but for most of molecular biology and biochemistry.

He was responsible for developing a system in which the mechanism of steroid action and the regulation of gene expression can be studied in continuously cultured mammalian cells, a system which is widely used in research laboratories today and which in recent years he extended to the selection and isolation of mutant cell clones with altered responses to steroid hormones and cyclic nucleotides. This approach is providing insights to many of the fundamental problems in endocrinology and is playing a significant role in the further acquisition of knowledge in fields of genetics, cellular and developmental biology, and cancer.

Equally important were his theoretical contributions to a variety of biological and medical fields. He introduced the idea that the regulation of the levels of specific proteins might occur at steps subsequent to the formation of their messenger RNA molecules. From a consideration of the problem of the transformation of a normal cell into a malignant or cancerous one with uncontrolled growth, he developed a unifying theory of growth regulation termed '*pleiotropy*' by which a normal cell, unlike its malignant counterpart, coordinates a diverse set of intracellular reactions to arrest its growth in response to extracellular

conditions. In his final theoretical contribution, he described a plausible mechanism to account for the evolution of hormonal regulation, that is characteristic of multicellular organisms, from simple unicellular organisms.

His impact on medical research has been generated not only by his training and education of numerous esteemed scientists and physicians throughout the world but also by the unusual excitement about science that he created among colleagues and acquaintances.

To those who knew him scientifically, his death is the loss of exciting ideas; to those who knew him musically, the loss of distinguished talent; to those who knew him personally, the loss of intellectual brilliance, extraordinary wit, great warmth and charm. To those who were fortunate enough to experience all of these various gifts, his death is the end of an era.

Reiji Okazaki, the Japanese molecular biologist, died on August 1 at the age of 44.

Okazaki arrived at the forefront of molecular biology in 1966 with his discovery that the synthesis of new DNA strands during the process of DNA duplication seems to occur in rather short sections (now known to everyone as '*Okazaki pieces*') that only later are joined together to make the long uninterrupted strands of the finished product. This finding explained how two new strands of opposite polarity could be synthesised at a single locus travelling in one direction along the parental double helix, and it therefore reconciled the properties of DNA polymerase with the large-scale structure of replicating DNA. Since then he had worked indefatigably to determine what is the direction of synthesis of the short pieces of DNA, how they are started and how finally they are joined together. These experiments involved a very high level of technical sophistication and were followed with great interest by all workers in the field. Okazaki was a school-boy in Hiroshima when the bomb was dropped and this may have been the cause of his death, from leukaemia—a young man still at the height of his powers. At the time of his death he was professor of molecular biology at Nagoya University in Japan.